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Radiation-induced peculiarities of cytochrome P-450IIE1 and oncogenes mRNA accumulation in different rat tissues

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Cytochrome CYP2E1 gene as well as c-fos and c-myc oncogenes expression have been studied in different tissues of Wistar rats kept at Chernobyl's exclusion zone. Reliable differences are revealed in above-mentioned genes expression on RNA level by comparing data obtained from experimental and control animal groups.

The connection between the accumulation of *c-fos* mRNA and P-450IIE1 mRNA (B2mRNA$_x$, CYP2E1 mRNA) during the prereplicative period of rat liver cells division has been shown in previous work [1]. Now we are interested in relationship between oncogenes (*c-fos* and *c-myc*) and CYP2E1 gene expression under radiation influence which takes place in Chernobyl's exclusion zone. The interest is due to the key role of both C-FOS, C-MYC and P-450IIE1 proteins in cell transformation [2, 3]. In this work the attempt has been done to study if any relationship takes place between the expression of these genes on RNA level after long action of low level radiation.

Materials and Methods. *Animals.* Two groups of albino rats (Wistar) that have common ancestry were bred at the animal care facilities of the Institute of Experimental Pathology, Oncology and Radiobiology at Kyiv and at Chernobyl's exclusion zone. Male rats were 4 months of age for all experiments reported here. The whole-body dose of radioactivity absorbed for this period by the rats living in Chernobyl through inhalation and feeding amounted to 1.2 ± 0.3 cGy for Cs^{137}. This is approximately 10 times higher than the dose absorbed by the control rats.

Tissue acquisition. Different tissue samples were used from the animals grown in Kyiv (control) and Chernobyl (irradiated). The livers, brains, spleens and testicles were removed, rinsed in phosphate-buffered saline (Na_2HPO_4, 1.5 g/l; NaCl, 8 g/l; $MgCl_2 \cdot 6H_2O$, 0.1 g/l; KH_2PO_4, 0.2 g/l; KCl, 0.2 g/l; $CaCl_2 \cdot 2H_2O$, 0.132 g/l) and frozen in liquid nitrogen. These tissues were maintained at −70 °C. All data points involved pooled tissue samples of each kind from 4—6 rats.

RNA isolation. Total RNA from the samples of irradiated and control animals was isolated using guanidine thiocyanate by a procedure [4] and quantified by measuring its absorbance at 260 nm; purity was assessed by determining the 260 nm/280 nm ratio. After electrophoresis on denaturing gels, RNA integrity and loading equivalence were assessed by ethidium bromide staining.

Northern blotting and hybridization. Samples of total RNA were electroforetically size separated in 2.2 M formaldehyde — 1 % agarose gels [5]. The RNA was transferred to Hybond-N membranes («Amersham», Great Britan) by a vacuum blotting method and crosslinked to the membranes using ultraviolet radiation. cDNA probes were randomly primed to specific activity of at least 10^8 cpm [6]. Prehybridization, hybridization, and washing processes were performed as described in [7]. Hybridized blots were exposed to X-ray film at −70 °C. Densitometer scans of autoradiographs were performed on an LKB Ultroscan XL Laser Densitometer. The valley-to-valley method was used to calculate baselines, and data were normalized to the sum of signals. All blots were

de-hybridized by incubation in H_2O at 90 °C for two minutes twice. They were checked for total removal of the probe by overnight exposure to X-ray film. Those blots showing total removal of the initial probe were then re-hybridized to a different cDNA clone.

cDNA clones. Probes were prepared from the following clones: 1-kb *PstI* fragment of *v-fos* [8]; the *SalI-PstI* fragment of *pv-myc* corresponding to *myc* third-exon [9]; 0.3-kb *PstI* fragment of CYP2E1 (cDNA of B2mRNA$_x$ [10]).

Results and Discussion. The experiments reported here were designed to determine whether the expression of different cellular oncogenes as well as cytochrome P-450 genes is altered in rats following the long exposure to radioactive substances absorbed by inhalation and feeding in Chernobyl's area. Previous work from our laboratory has shown that the dynamics of accumulation of *c-fos* mRNA is strongly related with that of P-450 2E1 mRNA during the prereplicative period of rat liver cells division [1]. Experiments by other groups have shown modulation of *c-fos* mRNA following exposure of cells in culture to X-rays or other DNA-damaging agents [11—13], possibly leading to cell transformation and neoplasia. Fig. 1 it was shown by the microdensitometry results for *c-fos* oncogene. These results demonstrate the significant reduction of *c-fos* RNA level in almost all the tissues of Chernobyl's animals relative to controls.

Fig. 2 represents the level of *c-myc* RNA in different tissues of experimental and control rats. These results demonstrate *c-myc* RNA accumulation in spleen and testicle cells. Expression of *c-myc* was somewhat reduced in liver relative to controls, while in brain it is essentially unaffected by the irradiation. This suggests that the exposure of animals to radioactive substances absorbed by inhalation and feeding causes different responses on the level of RNA accumulation in different cell types, as well as selective modulation of specific oncogenes. It should be noted that the changes in expression observed here are relatively small. This is in keeping with experiments from other groups demonstrating only moderate gene modulation following exposure to DNA-damaging agents [11, 14—16]. Since increased expression of some oncogenes (including *c-myc*) has been shown to play a role in cellular transformation [1], our results also suggest that one mechanism of irradiation-mediated oncogenesis may involve the modulation of oncogene expression in selected tissues. On the other hand, it is of great importance the decrease in activity of detoxification cell systems [17] apparently caused by the damage of detoxifying enzymes as well as their genes or mRNAs thus enhancing the negative action of radiation on cell structures. For instance, it was recently demonstrated that low level radiation (LLR) represses enzymatic activity of superoxide dismutase [18], glutathione transferase and glutathione reductase [19] in human tissues. Our preliminary results also revealed some repression of activity of a microsomal enzyme P-

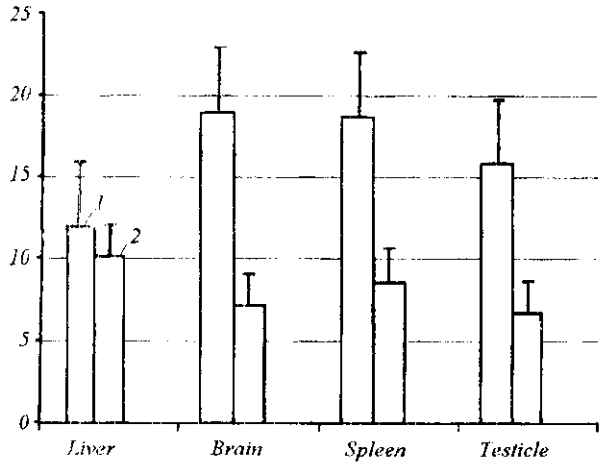

Fig. 1. Analysis of *c-fos* RNA levels in different tissues of control and irradiated animals. All microdensitomrtric data were normalized to the sum of signals: *1* — Kyiv, *2* — Chernobyl

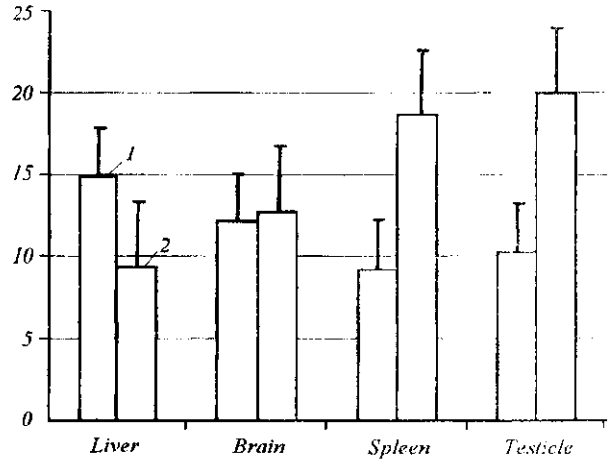

Fig. 2. Analysis of *c-myc* RNA levels in different tissues of control and irradiated animals. All microdensitomrtric data were normalized to the sum of signals: *1* — Kyiv; *2* — Chernobyl

450IIE1 in rat liver under LLR influence (the data not shown). At first, the last enzyme attracted our attention during the investigation of the early period of rat liver regeneration caused by partial hepatectomy (PH). It was revealed that at the early hours of regeneration the accumulation of messenger RNA for protooncogene *c-fos* and gene of P-450IIE1 undergo sharp fluctuations but in reciprocal manner [1, 20]. The gene CYP2E1 is a member of cytochrome P-450 gene superfamily (CYP) encoding a group of hemoproteins that mostly catalyze the oxidative metabolism of hydrophobic endogeneous compounds like steroids, fatty acids, prostaglandins and exogenous chemicals including drugs, carcinogens and environmental pollutants [21]. These substrates can be converted either to inert polar metabolites further eliminated in a water-soluble form or to cytotoxic or carcinogenic derivatives. Possible correlation of P-450IIE genes with malignancy was noted in some investigations [3, 22,]. Therefore it is not surprising that under the influence of radiation the expression of gene CYP2E1 essentially changes, as Fig. 3 shows. The RNA levels for this gene are very low in brain, spleen, and testicles. That is in accordance with the previously revealed liver-specific expression of CYP2E1 [23]. But there is obvious discrepancy between the increasing of RNA level and reduction of enzymatic activity of this cytochrome in liver under LLR. We suppose, it may be due to the radiation-induced mutations of this gene, as well as other damages on the way from DNA to protein.

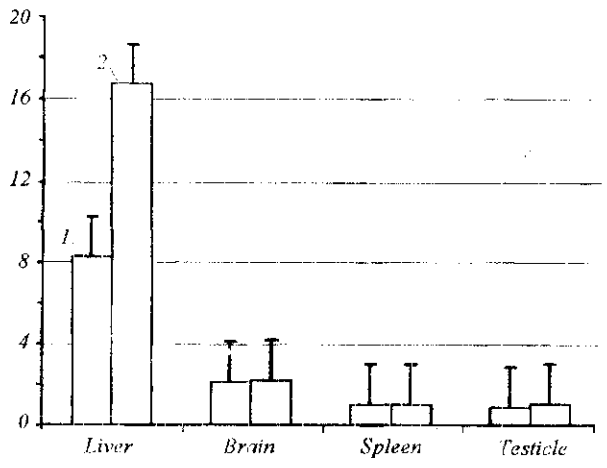

Fig. 3. Analysis of CYP2E1 RNA levels in different tissues of control and irradiated animals. All microdensitometric data were normalized to the sum of signals: *1* — Kyiv; *2* — Chernobyl

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Индуцированные радиацией тканеспецифические особенности содержания мРНК цитохрома P450IIE1 и онкогенов у крысы

Резюме

Вивчали експресію гена цитохрому CYP2E1, а також онкогенів c-myc і c-fos у різних органах щурів лінії Wistar, що утримувалися в умовах Чорнобильської зони відчуження. При порівнянні даних для дослідних і контрольних груп тварин виявлено вірогідні розбіжності в експресії вказаних генів на рівні РНК

В. И. Прима, Л. Г. Глушакова

Індуковані радіацією тканиноспецифічні особливості вмісту мРНК цитохрому P450IIE1 та онкогенів у щура

Резюме

Изучалаи экспрессию гена цитохрома CYP2E1,а также онкогенов c-myc и c-fos в различных органах крыс линии Wistar, содержавшихся в условиях Чернобыльской зоны отчуждения. При сравнении данных для опытных и контрольных групп животных выявлены достоверные различия в экспрессии указанных генов на уровне РНК.

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