basic exposure was to thiotepa, except in the case when preliminary treatment was given with thiotepa and the number of chromosomal breaks was significantly reduced mainly on account of a fall in the level of chromatid (single) breaks, and in the version in which preliminary treatment with dipin was given and the decrease in the number of chromosomal breaks took place as a result of a decrease in the number of paired breaks. The results of the experiments indicate clearly that a combination of dipin and thiotepa leads to the appearance of an effect of crossed "clastogenic adaptation."

The following hypothesis can be put forward on the basis of analysis of the results. Despite differences in the mechanisms of formation of chromosomal aberrations, both dipin and thiotepa can weaken each other's mutagenic effect due to the phenomenon of "crossed clastogenic adaptation," but at the same time the character of the mutagen which was used for preliminary treatment affects the predominant repair of single or paired breaks. This fact suggests that the preliminary concentration not only activates a set of repair enzymes, but also "tunes" the repair system for eradication of injuries typical of that particular mutagen. For the "crossed clastogenic adaptation" effect to arise between two mutagens, it is evidently essential that similar types of lesions be found in the spectrum of chromosomal aberrations induced by these mutagens.

LITERATURE CITED

1. B. Kaina, Mutat. Res., 111, 341 (1983).
2. R. Rieger, A. Michaelis, and S. Takehisa, Mutat. Res., 144, 171 (1985).

CHANGES IN GENE EXPRESSION IN THE RAT BRAIN INDUCED BY ZAJDELA'S ASCITES HEPATOMA

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The development of a malignant neoplasm is accompanied by progressive and varied disturbances of homeostasis, which even affect such a "protected" organ of the body as the brain. The writers showed previously that changes in carbohydrate and lipid metabolism in rat brain tissue are among the principal manifestations of the "distant" action of transplantable hepatomas H-27 and ZAH [3]. The possibility cannot be ruled out that weakening of the functional capacity of the brain regulating centers (especially those controlling the internal medium of the body), arising as a result of this action may take a contribution to the formation of metabolic "vicious circles" leading ultimately to death.

The aim of this investigation was to study expression of certain genes in the brain of animals with a rapidly growing transplantable Zajdela's ascites hepatoma (ZAH; the animals survived for 5-6 days).

The test objects were the genes of actin (a constitutively expressed "household" gene, the c-Ha-ras protooncogene conjecturally coding for G-proteins involved in signal transmission from a surface receptor to the internal medium of the cell [5]; the "stressor" hsp70 gene, induced by various unfavorable factors and, in particular, by heat shock (and, consequently, by a febrile state), by toxic compounds, and disturbances of the energy supply [6, 9, 10], i.e., by factors whose existence can be regarded as perfectly feasible in a tumor-bearing organism.

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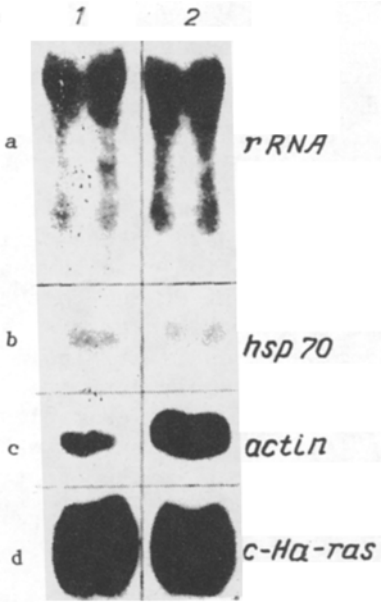

Fig. 1. Blot hybridization of brain RNA with molecular probes, for mRNA of heat shock protein hsp70 (b), mRNA of actin (c), and mRNA of protooncoprotein c-Ha-ras (d). Lane 1) Brain RNA of healthy animal; lane 2) brain RNA of animal with ZAH hepatoma. Posthybridization staining of filter with methylene blue to reveal 28S and 18S rRNA shown above (a).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 140-180 g: intact rats and recipients of ZAH. The ZAH cells were inoculated intraperitoneally and the animals were killed on the 5th day of development of the tumor. After decapitation the brain tissue was removed, frozen in liquid nitrogen, minced, and solubilized in 5 M guanidine isothiocyanate. Subsequent isolation of total cell RNA, including centrifugation in a CsCl gradient, was carried out in accordance with the known recommendations [2]. Electrophoresis of RNA under denaturing conditions, its transfer to nylon filters (Hybond-N, "Amersham International"), prehybridization, and hybridization with labeled ^{32}P, in the nick-translation reaction with molecular probes, washing of the filters, and autoradiography, were carried out as described previously [1, 4]. The quantity of RNA transferred to the filter was determined by the degree of staining with methylene blue [2].

As molecular probes we used plasmid pDP8 with cDNA insert (1750 b.p.), human gene hsp70 in the EcoRI-SalI site of the pIS13 vector (G. P. Thomas, England); plasmid A2-1.8H[3], containing 1.8 kbp and 1.6 kbp HindIII, fragments of the actin gene of clone λDm A2 in the HindIII pBR322 site [8]; clone Bs9 of the Ha-MuSV oncogene measuring 0.49 kbp in the EcoRI pBR322 site [7].

EXPERIMENTAL RESULTS

The stimulus which prompted this investigation was data obtained previously on tumor-induced changes in carbohydrate and lipid metabolism in the brain tissue of tumor-bearing animals [3]. The system of "stressor" genes [6, 9, 10], capable of being activated as a result of exposure of the cell to a wide range of different unfavorable factors, and whose activation is coupled with inhibition of expression of other hitherto transcribed genes, arouses particular interest in this connection.

The results of blot-hybridization of RNA from the brain of intact animals (lane 1) and animals with ZAH (land 2) with DNA-probes for "stressor" gene hsp70 (b), the gene of actin (c), and protooncogene c-Ha-ras (d) are shown in Fig. 1. Expression of the hsp70 gene (the main representative of the family of "stressor" genes) not only was not strengthened, as might have

been expected in view of the natural suggestion that changes taking place in the internal medium of the body were "stressor" in character, but, on the contrary, was appreciably inhibited in the brain of a tumor-bearing animal (in the top part of Fig. 1 a filter stained with methylene blue is shown, in which the 28S and 18S rRNA can be seen, so that the intensity of the hybridization signal can be correlated with the amount of RNA transferred to the filter). It should be noted that, judging by our data (not shown here), constitutive expression of hsp-70 in the brain of normal rats is very intensive and exceeds that in all other tissues studied (liver, spleen, muscles, kidneys, thymus). In the absence of inducers of the hsp70 gene (heat shock, hypoglycemia, transition metals, inhibitors of energy metabolism, analogs of amino acids, steroid hormones, growth factors, etc.) [6, 9, 10], its expression in the cells as a rule is at a low level. High activity of this gene in the brain may be evidence that in this case hsp70 performs certain special and as yet unknown functions.

The other gene (of actin) studied in this investigation is expressed constitutively in all tissues and belongs to the number of so-called "housekeeping genes," the functions of which are essential for normal vital activity of the cell. The reaction of this gene to growth of ZAH in the body differs from that of the hsp70 gene and is expressed as marked stimulation of its expression (Fig. 1c). This state of affairs is evidence that transcription changes in the brain tissue of animals with ZAH are in different directions.

The c-Ha-ras protooncogene has been the subject of numerous investigations because of its special role in the process of malignant transformation of cells; the activated form of this gene is found more frequently than others in "spontaneous" human tumors. According to existing views, c-Ha-ras codes for a protein belonging to the G family, responsible for transmission of mitogenic signals from surface receptors into the internal medium of the cell [5]. As will be clear from Fig. 1d, the reaction of this gene to the development of ZAH is intermediate between the reactions of the two genes studied previously.

Thus the expression of two genes with different functions and different mechanisms of regulation is modified (although to different degrees) in the brain of animals with ZAH. It is quite probable that these opposite and, in some cases substantial changes may make a contribution to the general imbalance and disturbance of homeostasis characteristic of a tumor-bearing organism. However, many problems remain unexplained and, in particular, the character of changes in gene expression in the tissues depending on the state of tumor development (with ZAH the process develops extremely quickly, and the changes found may therefore differ from those which perhaps take place in slowly growing tumors); factors mediating the effect of the tumor on gene expression in distant tissues, and so on.

These problems will be topics for experimental study.

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LITERATURE CITED

1. A. V. Likhtenshtein, V. L. Moiseev, and M. M. Zaboikin, Mol. Biol., No. 6, 1513 (1987).
2. E. F. Fritsch, T. Maniatis, and J. Sambrook, Molecular Cloning of Eukaryotic Genes, Cold Spring Harbor, New York (1982).
3. V. A. Chekulaev, V. P. Shelepov, G. R. Pasha-zade, and V. S. Shapot, Biokhimiya, 52, No. 9, 1501 (1987).
4. V. P. Shelepov, G. R. Pasha-zade, V. A. Chekulaev, et al., Byull. Éksp. Biol. Med., No. 11, 612 (1987).
5. M. J. Berridge, Annu. Rev. Biochem., 56, 159 (1987).
6. R. H. Burdon, Biochem. J., 240, 313 (1986).
7. R. W. Ellis, D. de Feo, T. Y. Shih, et al., Nature, 292, 506 (1981).
8. E. A. Fyberg, K. L. Kindle, N. Davidson, and A. Sodja, Cell, 19, 365 (1980).
9. L. Nover, Heat Shock Response of Eukaryotic Cells, ed. by L. Nover, Berlin (1984), pp. 7-11.
10. H. R. B. Pelham, Cell, 46, 959 (1986).