CYTOGENETIC CHARACTERISTICS OF LUNG TUMORS INDUCED IN BALB/c MICE BY TRANSPLACENTAL ACTION OF N-NITROSOETHYLUREA

A. Ya. Likhachev

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A cytogenetic study of adenocarcinomas of the lung in BALB/c mice exposed to the transplacental action of N-nitrosoethylurea showed that 121 of 145 metaphases studied contained 40 chromosomes. Meanwhile individual hypodiploid, hyperdiploid, and tetraploid cells were found. In all the cells the chromosomes had the ordinary telocentric structure. The question of possible changes in karyotypes of the tumors studied, which cannot be detected by ordinary methods of chromosome staining, is discussed.

KEY WORDS: tumors of the lungs; N-nitrosoethylurea; chromosome set.

Evidence of the high susceptibility of mice to the transplacental action of carcinogens has recently been published. It is important to note that, despite considerable difference in the experimental conditions under which carcinogenic substances of different chemical classes and mice of different strains were used, tumors developed mainly in the lungs of these animals [7, 9]. In particular, mouse lung tissue is very highly sensitive to the transplacental carcinogenic action of N-nitrosoethylurea (NEU) [7]. However, no investigations have yet been undertaken for the cytogenetic study of tumors developing as a result of the transplacental action of carcinogens. Yet this model of lung tumors in mice has obvious advantages, for these tumors (adenomas and adenocarcinomas) arise in nearly all animals even after a single exposure to NEU. During embryogenesis and early postnatal development, the lung tissue is suitable for cytogenetic study because of the large number of dividing cells; it is accordingly a convenient object with which to study the early responses of the chromosomal apparatus to the transplacental action of carcinogens. The property of inducing tumors in this situation by means of a wide variety of carcinogens also enables a comparative cytogenetic study of these substances to be made.

The object of the present investigation was to study the cytogenetic characteristics of lung tumors induced in mice by the transplacental action of NEU.

EXPERIMENTAL METHOD

Lung tumors arising in the progenies of the first generation of BALB/c mice receiving a single intraperitoneal injection of an aqueous solution of NEU in a dose of 20 mg/kg on the 17th-18th day of pregnancy were used. A cytogenetic study was made of 11 mice with lung tumors, but metaphase plates of satisfactory quality were obtained only from four mice. The tumor tissue was carefully separated from normal lung tissue and dried chromosomal preparations were made by the usual method and stained with azure-eosin.

EXPERIMENTAL RESULTS

All four lung tumors used for cytogenetic analysis were adenocarcinomas with a papillary structure (Fig. 1). The mice in which these tumors developed were killed on the 327th, 329th, 342nd, and 351st days of postnatal life, respectively. Altogether 145 metaphases were analyzed (Table 1).

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TABLE 1. Distribution of Cells by Number of Chromosomes in Lung Adenocarcinomas Arising in BALB/c Mice as a Result of the Transplacental Action of NEU

No. of tumor	Number of metaphases analyzed	Number of chromosomes											
		31	34	37	38	39	40	41	42	43	44	79	80
1	20	—	—	—	1	—	19	—	—	—	—	—	—
2	71	1	—	—	3	3	56	3	1	1	1	1	1
3	8	—	—	—	—	—	7	—	1	—	—	—	—
4	46	—	1	1	2	1	39	1	—	—	—	1	—
Total	145	1	1	1	6	4	121	4	2	1	1	2	1

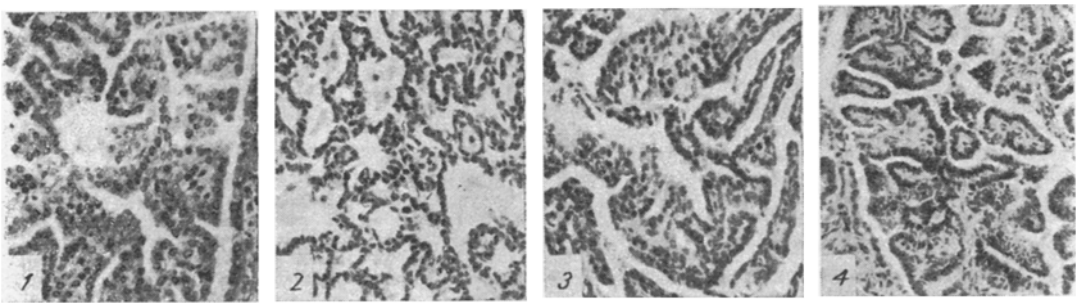

Fig. 1. Microscopic structure of lung tumors (Nos. 1-4) in BALB/c mice used for cytogenetic analysis. All tumors are papillary adenocarcinomas. Stained with hematoxylin–eosin, 200×.

Normal mouse dipoloid cells contain 40 telocentric chromosomes [3]. As Table 1 shows, the modal class in all the tumors studied was also formed by cells with 40 chromosomes; these cells with a diploid set of chromosomes, moreover, accounted for over 83% of the total number of metaphases studied. Characteristically in all four tumors, cells of this type were found with virtually the same frequency. Hyperdiploid cells were found much less frequently (Table 1). For instance, single metaphases consisting of 41–44 chromosomes were found, but most often they contained 41 chromosomes. Three metaphases were tetraploid or near tetraploid (Fig. 2). Hypodiploidy also was observed in some cells, and in this case the commonest numbers of chromosomes in these metaphases were 38 and 39. Cells containing 31, 34, and 37 chromosomes were found in different tumors (Table 1). The chromosomes in all the cells studied had the usual telocentric structure.

The cytogenetic study of lung adenocarcinomas induced in BALB/c mice through the transplacental action of NEU thus showed that all tumors had diploid set of chromosomes with a modal class of 40, and with very slight variations toward both hyper- and hypoploidy. In the latter case the loss of some of the chromosomes during preparation of the specimens cannot be entirely ruled out and the hypodiploidy described may have been an artefact in at least some of the cells.

By the ordinary methods of studying mouse chromosomes their identification is not always possible because of the special features of the chromosome set. By the method used to karyotype the chromosomes of certain tumor cells it was impossible to detect changes in the karyotype of diploid tumor cells or to reliably identify the additional chromosomes in the hyperdiploid sets or the absent chromosomes in the hypodiploid sets. It will be recalled that, according to Bayreuther [2], most primary mouse tumors had the normal karyotype, and according to Pogosyants et al. [1], this is also a characteristic feature of primary tumors of the striped hairy-footed hamster. A normal diploid karyotype is thus also characteristic of lung tumors induced in mice by the transplacental action of NEU, despite the fact that all the tumors studied were malignant (adenocarcinomas).

However, on the basis of the foregoing facts the possibility of deviations from normal in the karyotype of the diploid tumor cells cannot be ruled out, for the chromosomes were stained in the ordinary way both in the

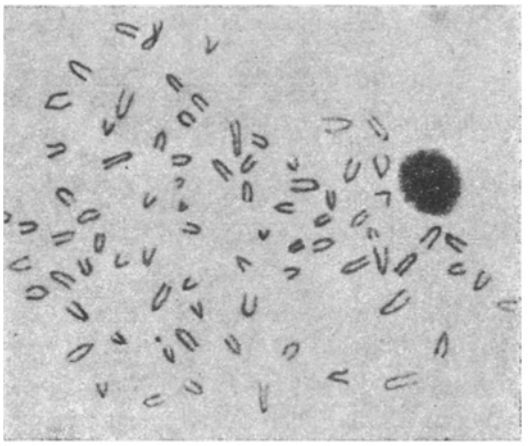

Fig. 2. Metaphase containing 80 telocentric chromosomes found in tumor No. 2 (stained with azure–eosin).

present investigation and in those cited above. The use of new methods of differential staining of chromosomes in the cytogenetic study of lung tumors induced in mice by the transplacental action of carcinogens will show whether in fact a diploid karyotype is actually characteristic of mouse lung adenocarcinomas. Recent findings indicate that differential staining of chromosomes can reveal changes in karyotype even in tumor cells in which no abnormality of the karyotype can be found by the usual staining methods [4, 5, 10].

A solution to this problem is particularly important because in recent years increasing evidence has been obtained in support of the hypothesis of the specificity of chromosomal changes in tumors, according to which particular changes in the chromosomes are characteristic of tumors induced by different agents despite the complete morphological identity of the tumors produced [6, 8].

Comparison of the karyotype of lung adenocarcinomas induced in mice by the transplacental action of different carcinogens would thus be of the greatest interest from this standpoint.

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