SPECIFIC MUTATION OF THE Ha-ras ONCOGENE IN SKIN TUMORS INDUCED IN MICE UNDER DIFFERENT EXPERIMENTAL CONDITIONS

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Activation of oncogenes of the ras family, connected with their point mutation, is found in various tumors of animals and man [1, 3]. In particular, in skin tumors induced in mice by the initiating action of 7,12-dimethylbenz(a)anthracene (DMBA) ana by the promoting action of 12-0-tetradecanoylphorbol-13-acetate (TPA), a specific mutation of the Ha-ras oncogene has been discovered with substitution of adenine (A) for thymine (T) in position 2 of the codon, leading to substitution of glycine for leucine in the corresponding protein product p21 [2, 4, 9]. The first results of this kind were apparently evidence of correlation of the presence of the mutant oncogene with the action of DMBA, on the one hand, and with the degree of malignancy, on the other hand [9, 13]. Further investigations in this direction, however, showed that in certain cases the mutant Ha-ras was not found in DNA from skin carcinomas induced by the combined action of DMBA and TPA [12]. The same mutation may also be present in papillomas induced in mice by prolonged application of TPA without initiation [8, 12]. It was shown previously that the transplacental action of DMBA not only initiates cutaneous carcinogenesis in the first generation progeny, but also increases sensitivity to promotion in the second generation also [5, 7], possibly in connection with transgeneration transmission of the initiating effect.

The aim of this investigation was to discover a specific mutation in the second position of the 61st codon of the Ha-ras oncogene in DNA isolated from skin tumors induced in mice with the aid of TPA under different initiation conditions, including testing the possibility of transgeneration transmission of the inducing effect.

EXPERIMENTAL METHOD

Altogether 31 skin tumors developing in 23 SHR albino mice (male and female), from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR, divided into four groups, were studied. The experiments were conducted at the Professor N. N. Petrov Research Institute of Oncology. Mice of Group 1 were the first generation progeny obtained by mating intact females with males which had received an intraperitoneal injection of ethylnitrosourea (ENU) In a dose of 80 mg/kg 2 weeks before mating. The mothers of the mice of Group 2 received DMBA (Fluka, Busch, Switzerland) in a dose of 100 mg/kg in aqueous-lipid emulsion [5], by intraperitoneal injection on the 21st day of pregnancy. Mice of Group 3 were born from crossing of mice of Group 2 with one another. Mice of Group 9 were the offspring of animals not subjected to any procedures. Starting with the age of 12 weeks, TPA was applied twice a week in a dose of 6.15 mg ("Sigma," USA) in 0.1 ml acetone to the skin of all the mice studied.

The mice with skin tumors were killed with ether vapor. Part of the tumor tissue was studied morphologically, in sections stained with hematoxylin and eosin. The rest of the material was frozen in liquid nitrogen for subsequent molecular-biological investigation, carried out at the International Agency for Cancer Research. To isolate DNA from the tumor tissue, an

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TABLE 1. Results of Analysis of Mutation in Position 2 of the 61st Codon of the Ha-ras Oncogene in Mouse Skin Tumors

Group	Procedure	Number of animals	Number of tumors studied and histologic diagnosis	Number of tumors with revealed mutation
1-	Application of ENU to father plus TPA	5	5 papillomas	0
2-	Transplacental action of DMBA	6	5 papillomas	3
			5 carcinomas	5
3-	Crossing mice of Group 2	4	4 papillomas	0
4-	TPA	8	12 papillomas	0

enzymic method with proteinase-K was used. The presence of a specific mutation in the second position of the 61st codon of the Ha-ras oncogene was determined by the method of detection of restriction fragment length polymorphism following restriction of the isolated high-molecular-weight genomal DNA (10 μg) by restriction endonuclease Xba I (BRL, USA) (the presence of the (A) for (T) substitution in the position analyzed leads to the appearance of an extra restriction site for this enzyme). The restriction fragments were separated by electrophoresis in 0.7% agarose gel and transferred to Genescreen Plus nylon membranes ("Dupont") in accordance with the Southern technique [11]. After prehybridization the filters were hybridized, using DNA of the viral oncogene Ha-ras, labeled with ^{32}P, as the probe. After hybridization, washing, and drying, the filters were exposed, using supersensitive MP x-ray film ("Amersham," England) for 3-4 days at —70°C with intensifying screens.

EXPERIMENTAL RESULTS

The morphologic study showed that the majority of tumors studied were papillomas. Malignant tumors (squamous-cell carcinomas) were observed only in the mice of Group 2 (Table 1). All skin tumors developed during the period from 30 to 42 weeks.

The results of the molecular-biological investigation showed that no mutation could be found in any single case of mice not exposed to the direct action of the inducing carcinogen. Meanwhile, in the group of animals receiving DMBA transplacentally, eight of the 10 skin tumors studied, including all five analyzed carcinomas, were positive for Xba I RFLP; in one animal with multiple skin tumors one papilloma was negative but the other three tumors (two carcinomas and one papilloma) were positive. The results demonstrate the existence of definite correlation between the presence of the (A) for (T) substitution in position 2 of the 61st codon of the Ha-ras oncogene, and, first, preceding exposure to the carcinogen DMBA and, second, the degree of differentiation of the tumor. Our results do not agree with those obtained previously by other workers, who showed that induction of the same specific mutation can take place in papillomas induced by a promotor without initiation [8, 12]. On the other hand, these differences may be explained by the relatively small number of tumors studied, although altogether 21 papillomas from 17 animals of three groups not subjected to direct initiation were analyzed, and on the other hand, the differences may perhaps be determined by differences between the strains of mice used in the experiments. The probability that such differences may have affected the result does exist, because genes of the ras family in some cases directly determine differences in the sensitivity of different strains to carcinogens, as has been shown in the case of lung tumors [6, 10].

Models of carcinogenesis, assuming transplacental action of a carcinogen or the transmission of a carcinogenic effect in generations, are very attractive from the point of view of study of the role of oncogenes in the mechanism of development of tumors, primarily because the systemic action of a carcinogen or a preexisting (as yet hypothetical, but repeatedly demonstrated) genetic effect create the grounds for development of tumors in different situations and of different tissue genesis. The present study is the beginning of a molecular genetic analysis of extensive tumor material obtained in chronic experiments. The A—T mutation in position 2 of the 61st codon of the Ha-ras oncogene is characteristic of the action of DMBA, but in the course of future investigations it is intended to give a detailed assessment of the different mutations of this family of oncogenes.

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