Progression of Tumor Histiotype during Mouse Hepatocarcinogenesis Associated with the Viable Yellow (A\(^{y}\)) Gene

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ABSTRACT

Increasing attention has been focused recently upon those factors in carcinogenesis that are responsible for the proliferation of initiated cells and the increasingly aberrant phenotype that they progressively manifest. The agouti locus allele A\(^{y}\) (viable yellow) has been shown to be associated with conditions which favor promotion of cells that have been initiated by a wide variety of causes, in many organs, but has not been previously associated with tumor progression in those systems. In the current study, the presence of the A\(^{y}\) gene in a strain of mice not normally predisposed to hepatocarcinogenesis, C57BL/6N was, for the first time, associated not only with much earlier appearance, but with progression of the histiotype of hepatic tumors, following neonatal administration of diethylnitrosamine. At 52 weeks, 28 C57BL/6N mice demonstrated 7 mouse liver tumors 0.5 cm or greater in diameter, all of more benign histiotype, without associated metastasis. The 31 C57BL/6N-A\(^{y}\) demonstrated 194 mouse liver tumors at that time, 22% of which were of malignant histiotype, 19% of which were associated with metastasis. This system would appear to offer the possibility of identifying the underlying mechanisms for components of the carcinogenic process.

INTRODUCTION

Many reports describe mechanisms by which exogenous agents might enhance proliferation of initiated cell populations, a process that has been broadly defined as a component of promotion (1–3). These promoters and other agents, including mutagens, have also been examined for their ability to evoke increasingly aberrant phenotypes in initiated, replicating cells, a process broadly defined as progression (4–6). However, more limited information is available as to the endogenous basis for a process broadly defined as progression. The agouti locus allele A\(^{y}\) mouse offers an advantage as a test animal in bioassay procedures that use the liver as a target organ. This represents a mouse with little or no spontaneous predisposition to hepatocarcinogenesis, with a predicted short lag period toward response to hepatocarcinogens.

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promoted, whereas smaller tumors and background liver were sampled en bloc. Each section was prepared for standard histological stains (hematoxylin and eosin; periodic acid-Schiff) and those from hyenas for γ-glutamyl transpeptidase. The lungs were formalin fixed and step sectioned to determine the presence of metastasis.

Tumor Types

In brief, the tumor types can be described as follows (15):

- MLT-I/II. The periphery is most frequently composed of small diploid basophilic cells with indistinct borders. The more central cells demonstrate cytoplasmic enlargement resulting from fat, glycogen, and a variety of inclusions. Occasional areas (of varying size) of trabecular structure were noted. The trabeculae, which are generally 1 cell thick (rarely 2 cells), are composed of cells cytologically similar to those of the bulk of the tumor. They “blend” with the more solid portions of the tumors. These tumors conform to those previously termed adenoma or type A.

- MLT-III/IV. This type of MLT may demonstrate the residual histiotype of MLT-I/II that has been replaced to varying degrees by cytologically malignant foci. These foci are most frequently composed of greatly thickened trabeculae, with cytological atypia and mitosis or solid foci of malignant cells. Tumors of the MLT-I/II or surrounding liver by these foci is often evident. Occasionally, the malignant tumor is composed entirely of cells of the more malignant cytology, configuration, and invasiveness. These tumors conform to those previously termed hepatocellular carcinoma or type B.

RESULTS

AFP (Table 1). That an elevation of AFP was a valid marker for MLT in DEN-treated C57BL/6N-Ayw mice was confirmed at 20 and 30 weeks. The first 6 mice that achieved a circulating level of AFP of 1 μg/ml or more demonstrated extensive tumorigenesis at sacrifice. The 5 MLT that were 0.5 cm or more in diameter at these times were identified histologically as typical MLT-I/II, as were all smaller tumors. The basophilic foci typical of this regimen (19) were present and numerous.

In general, the timing and incidence of a significantly elevated, circulating AFP level was similar for treated C57BL/6N and B6C3F1 animals that previously reported (7, 15, 16). In the current experiments, MLT appeared somewhat later in C57BL/6N mice, whereas those in B6C3F1 mice appeared somewhat earlier than in the previous report. These variations in response emphasize the problems in using historical controls.

DEN-treated C57BL/6N-Ayw mice demonstrated elevated AFP values as early as 20 weeks of age, and an even higher incidence than did B6C3F1 mice at all time points. The differences between this strain and its progenitor strain C57BL/6N were highly significant (P < 0.001) at all time points.

MLT (Table 2). Of the 28 treated C57BL/6N mice killed at 52 weeks of age, 20 demonstrated tumorigenesis; only 6 (21%) had MLT greater than 0.5 cm in diameter and extensive tumorigenesis (4.5/liver). A total of 180 MLT of this size were examined, of which 25 (14%) were classified as MLT-III/IV. Of the 15 mice that demonstrated MLT of the more advanced histiotype, 4 (27%) demonstrated microscopic foci of metastasis in their lungs.

Of the 31 treated C57BL/6N-Ayw mice killed at 52 weeks, all had MLT greater than 0.5 cm in diameter and extensive tumorigenesis. A total of 194 MLT of this size were examined (6.3/liver), of which 49 (25%) were classified as MLT-III/IV. Of the 18 mice that had MLT of more-advanced histiotype, 4 (22%) demonstrated microscopic foci of metastasis. The number of tumors per liver for each strain of mouse was analyzed by the Kruskal-Wallis test. From these data, a highly significant value (P < 0.001) was obtained when C57BL/6N-Ayw and B6C3F1 mice were compared with C57BL/6N mice, but there was no significant difference between the former.

Eighteen untreated C57BL/6N-Ayw mice have been followed for 24 months without demonstrating any elevation of AFP. Six others were killed between 20 and 22 months, and the livers were examined to determine if any evidence of spontaneous alterations could be found. No gross or histological alteration was identified. Two more have been killed at 2 years and no lesions were identified. The remainder are still being followed.

DISCUSSION

The results of this study confirmed that the viable yellow (Ay) allele at the agouti locus acts as a “pan-promoter.” By this term, I suggest that it is capable of enhancing the carcinogenic process in many organs, subsequent to various initiating events. In prior studies, spontaneous (without known initiator), (20), viral (8-10), and chemically initiated (11, 12) carcinogenic sequences have been accelerated by the presence of these genes. The current study adds chemically initiated liver tumors to this list. The basis for this promoting effect is as yet unknown. The yellow mutations at the agouti locus affect a remarkably broad array of physiological processes. These include alterations in body weight and growth (21, 22); hormonal influences (23, 24); immunological aberrations (25, 26); and other (27, 28). Wolff, who has made a number of contributions to our understanding of Ay influence, has offered the suggestion that these genes have a systemic effect (29) which ultimately alters the microenvironment of the initiated cells (30). For the moment, however, no definitive identification of the mechanisms has been reported.
Evidence has been advanced that the major action of A" is to increase growth of the premalignant populations, without an increased rate of transformation to the malignant phenotype (10, 12). This suggests that the effect of the A" gene is mainly to increase the cell population of risk for the spontaneous, stochastic event(s) that results in the cancer phenotype (4, 31). This concept has been supported by the presence of tumors of identical histotype in A"-bearing and nonbearing mice. This sequence is remarkably close to that of previous proposals for more complex systems using carcinogens, promoters, and/or mutagens (4, 32, 33).

For the first time however, the presence of the A" gene has been shown to effect a significant progression in tumor cell populations to more malignant phenotypes. Thus, the histotype that I have designated as MLT-III/IV which is associated with significantly higher rates of invasion (13, 15), transplantability and metastasis (7, 15), was seen in highly significant numbers at a very early time. In a previous study wherein mice of the progenitor strain C57BL/6N were similarly treated with DEN, not one tumor of more advanced phenotype was seen as late as 70 weeks in the 180 MLT examined (7). Further, in that study, although exposure to phenobarbital shortened the lag time until appearance of MLT, no significant progression occurred at 1 year. In contrast, when mice genetically predisposed to tumorigenesis, B6C3F1, were similarly treated they consistently demonstrated a similar, albeit somewhat lesser, progression as occurred in the C57BL/6N-A". It was suggested that this progression in B6C3F1, mice might result from the effect of the carcinogen upon a genetically predisposed cell. If correct, this raises 2 possible explanations for the current findings: (a) that in this circumstance, the A" gene is related to a genetic predisposition to hepatic tumors; and (b) that tightly linked genetic material, transferred with the A" during development of the congeneric lines was that predisposing factor.

The failure of the presence of A" in prior studies to result in spontaneous hepatic tumors or progression except when "initiation" has occurred militates against this possibility. We have now carried untreated C57BL/6N-A" mice for more than 24 months without evidence of MLT or histologically demonstrated foci of altered hepatocytes. Further, it would be difficult to relate this phenomenon to the small aliquot of C3H genetic material transmitted with the A" during development of the congeneric lines was that predisposing factor.

An aspect of the A" effect that might be important in this hepatocarcinogenic system has been evidence that its presence is correlated with a more rapid growth of cells with a fully expressed, malignant phenotype (29). One could construct a scenario in which malignant cells possessing low growth rate potential exist in the less malignant MLT. Their stimulation by A" would result in earlier detection. However, no acceptable explanation for this phenomenon of progression is yet available.

Wolff has suggested that the presence of the A" gene could be a valuable component of a mouse bioassay system (34, 35). I would extend this suggestion to specifically identify the C57BL/6N-A" as an optimal mouse for that purpose. Although it becomes obese, its mortality through 24 months of observation does not differ from that of its progenitor. It appears to retain the resistance of the C57BL/6N to spontaneous tumors, since we have detected none in over 27 male and female mice followed for at least 24 months. And lastly, as is evident in this study, it can make manifest a tumor in a relatively short period of time after appropriate initiation.

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REFERENCES


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