Pergamon

European Journal of Cancer, Vol. 35, No. 13, pp. 1842–1845, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(99)00224-5

Original Paper

Chemical Hepatocarcinogenesis in Transgenic Mice Overexpressing Mature TGFβ-1 in Liver

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The role of transforming growth factor beta 1 (TGF-β1) in carcinogenesis is a controversial issue. Certain results suggest a promoter role of this growth factor whilst in other experimental models TGF-β1 seems to inhibit the process of tumorigenesis. In an attempt to resolve this problem, we have performed chemical hepatocarcinogenesis experiments on transgenic mice expressing a high level of active TGF-β1 in their liver. Transgenic production of TGF-β1 did not result in spontaneous tumour formation during our observation period. However, two carcinogens, thioacetamide and N-OH acetylaminofluorene, were more potent in transgenic than in wild-type mice, whereas aflatoxin B1 was equally effective in both groups. Our observations suggest that an increased level of TGF-β1 in the liver does not provide protection against the effect of chemical carcinogens. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: thioacetamide, transforming growth factor beta, mice, transgenic, carcinogens, aflatoxin B1, 2-acetylaminofluorene
Eur J Cancer, Vol. 35, No. 13, pp. 1842–1845, 1999

INTRODUCTION

CANCER IS fundamentally a genetic disease since the basic changes occur in the DNA. However, the manifestation of the disease is influenced by extrinsic factors such as the immune system, proliferative stage, etc. The cell surface growth factor/receptor systems convey important messages from the microenvironment to the nucleus, they can, therefore, influence the process of carcinogenesis. An important example of such growth factor/receptor systems is the TGF-β family, for review see [1]. TGF-β1 is the most extensively studied member of this family, it is one of the most potent inhibitors of epithelial cell proliferation, yet increased expression of TGF-β1 is frequently observed in experimental tumour models and in human tumours. The question of whether TGF-β1 has a protective or promoting role in carcinogenesis has not been resolved [2–5]. To address this question, we have conducted chemical hepatocarcinogenesis experiments on transgenic mice carrying a TGF-β1 transgene driven by an albumin enhancer/promoter. TGF-β1 facilitated the effect of two hepatocarcinogens, but failed to influence another hepatocarcinogen under our experimental conditions.

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Received 1 Jun. 1999; revised 4 Aug. 1999; accepted 18 Aug. 1999.

MATERIALS AND METHODS

Albumin/TGF-β mice were prepared as previously described [6]. In brief, fertilised eggs from F1 mice (C57Bl/6J×CBA) were microinjected with a cDNA of the murine albumin promoter and enhancer linked to a porcine TGF-β1 construct and the 3′ region of the human growth hormone gene which contains a polyadenylation signal. Multiple founder animals were obtained and four lines were established. Line 18 was bred to homozygocity and male mice from this line were used for the present experiments. This line had a 4–5-fold increased, stable plasma TGF-β1 level [7] compared with offsprings of F1×F1 (C57Bl/6J×CBA) crosses which were used as control mice. The genotype of the transgenic mice was checked by using PCR as previously described [7], however the serum TGF-β1 level was not measured in the present experiment.

The following carcinogen regimens were used: (1). Thioacetamide 300 mg/l in drinking water from 4 weeks of age till sacrifice [8]. (2) N-hydroxy-2-acetylaminofluorene (N-OH-AAF) 25 μg/g bodyweight intraperitoneally (i.p.) for 15

consecutive days from 3 weeks of age [9]. (3) Aflatoxin B1 6 μg/g bodyweight i.p. at 7 days of age [10].

The mice were sacrificed at timepoints indicated in Table 1 and thorough autopsies performed. Liver samples were fixed in 10% formaldehyde embedded and cut traditionally. The haematoxylin and eosin (H&E) stained sections (Figure 1) were diagnosed based on the criteria described by Frith and Ward [11]. If more than one lesion occurred in a liver, the animal was grouped according to the most severe diagnosis. The tumour incidence in the wild-type and transgenic mice was compared statistically using the χ^2 test. The animal study protocols were conducted according to the NIH guidelines for animal care.

RESULTS

No tumours occurred in the transgenic or wild-type mice during the 12 months observation period, i.e. increased TGF-β1 production did not change the spontaneous tumour rate in this strain of mice (Table 1). In contrast, in the event of liver carcinomas being induced by N-OH-AAF or thioacetamide, carcinogenesis was accelerated by TGF-β1, with higher incidence of carcinomas and adenomas observable in the transgenic mice. Aflatoxin B1 induced a low number of liver lesions in both transgenic and wild-type mice, but the difference between the two groups was not statistically significant.

Histologically, no differences were found between the tumours induced in transgenic or wild-type mice. Most of the carcinomas corresponded to typical trabecular hepatocellular carcinoma (HCC), occasionally showing focal pseudoglandular pattern (Figure 1). Primitive oval cell proliferation was sometimes present around and within the tumours in each experimental group. There was advanced cirrhosis with extensive intestinal metaplasia together with the proliferation of ductular cells in the thioacetamide-treated livers. Large eosinophilic cytoplasmic inclusions were frequently present in the N-OH-AAF, but rarely in the thioacetamide-induced carcinomas. No distant metastases or other primary tumours were observed in any of the study groups, but tumour cell emboli were occasional observations in the blood vessels of the livers irrespective of whether they derived from transgenic or wild-type mice.

DISCUSSION

In the present study, chemical hepatocarcinogenesis experiments were performed on TGF-β1 producing transgenic and wild-type mice. Two carcinogens (N-OH-AAF and thioacetamide) were more potent in the transgenic mice whilst aflatoxin B1 was equally effective in both groups.

There has long been an ongoing debate about the role of TGF-β1 in carcinogenesis. Sieweke and colleagues [12] reported on tumour formation in Rous sarcoma virus infected chickens, following TGF-β1 administration. Enhanced TGF-β1 expression was found locally in mouse skin, upon the effect of 12-tetradecanoyl-phorbol-13-acetate (TPA) tumour promoter treatment [13]. The tumour promoting effect of wounding has also been explained by local TGF-β1 production [14]. These and other data are supportive of the fact that TGF-β1 promotes the process of tumorigenesis. On the contrary, Glick and colleagues [4] reported that the loss of expression of TGF-β1 in skin is associated with a high risk of malignant conversion. Similar observations were made by Böttinger and colleagues [5] in transgenic mice, overexpressing a dominant negative mutant type II TGF-β receptor. Increased sensitivity for dimethylbenzanthracene (DMBA) was observed in the tissues (lung, breast) where the transgene was expressed.

There is also contradiction in the field of hepatocarcinogenesis. Earlier we reported an increased TGF-β1 expression and proposed an endogenous promoter role for TGF-β1 in rat chemical hepatocarcinogenesis [2]. Braun and colleagues [15] reached similar conclusions in another experimental system. The promoter role would not be surprising for an antiproliferative growth factor since many liver tumour promoters, e.g. 2-acetylaminofluorene (AAF) [16], phenobarbi-

Table 1. *Liver tumour incidence in Alb/TGFβ transgenic mice*

Carcinogen	Time from the start of carcinogenic treatment (months)		Total No. of mice	No. of mice with focus	No. of mice with adenoma	No. of mice with HCC	No. of mice with no lesions
Spontaneous	12	Transgenic	19	3	0	0	16
		Wild-type	9	0	0	0	9
NOH-AAF	12*	Transgenic	26	3	7	7	9
		Wild-type	10	1	1	0	8
	18*	Transgenic	7	0	1	6	0
		Wild-type	7	0	1	2	4
Thioacetamide	7*	Transgenic	13	0	5	6	2
		Wild-type	8	0	1	0	7
	9*	Transgenic	9	0	0	9	0
		Wild-type	10	0	6	4	0
Aflatoxin B1	12	Transgenic	12	2	2	1	7
		Wild-type	11	1	3	0	7

*χ^2 test $P<0.05$.

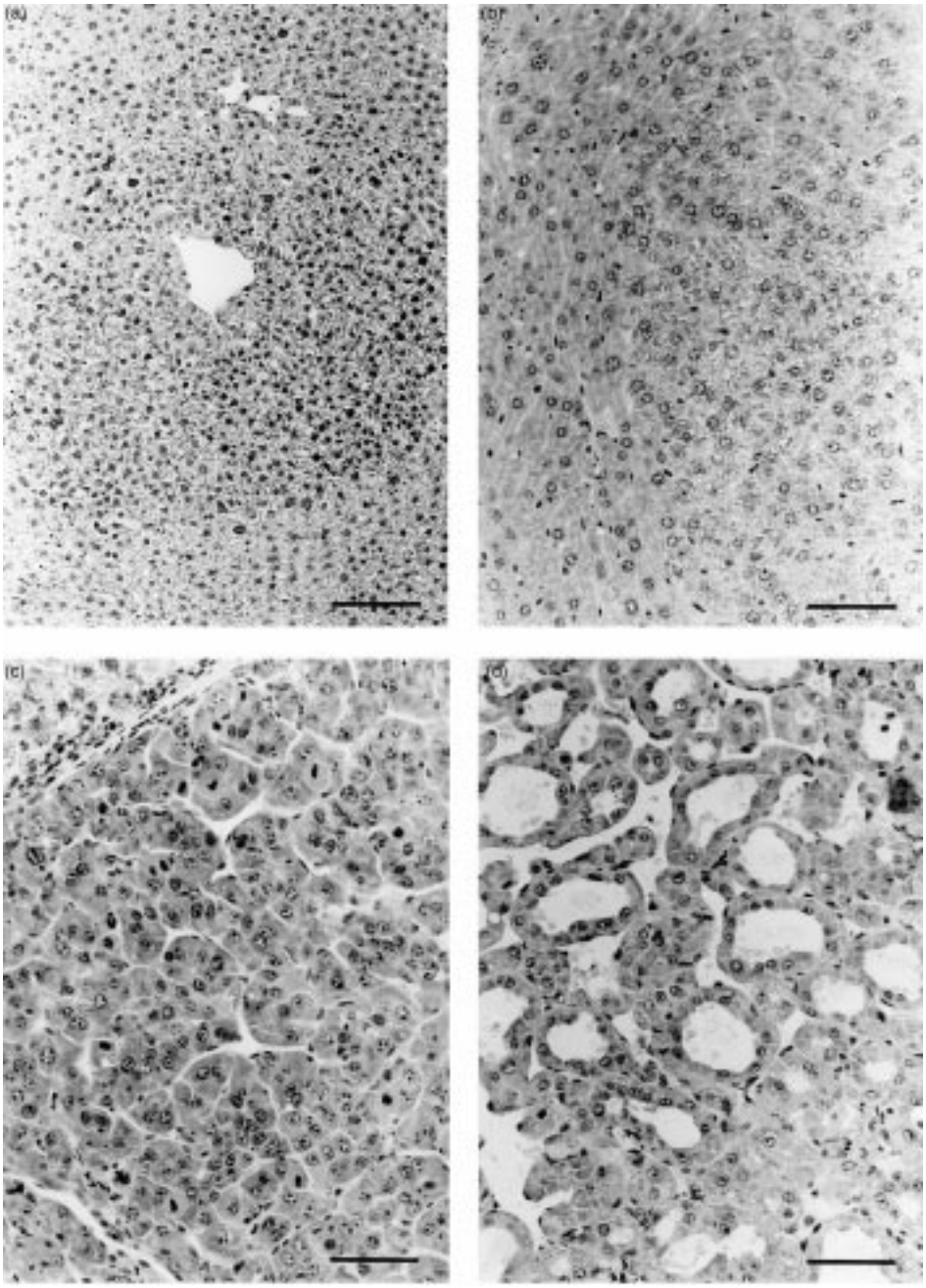

Figure 1. (a) Focus in an N-OH-AAF treated wild-type mouse liver. (b) The border zone of an aflatoxin B1-induced adenoma in a transgenic mouse liver. (c) Trabecular hepatocellular carcinoma (HCC) from a thioacetamide-treated transgenic mouse. (d) Pseudoglandular HCC from a N-OH-AAF-treated transgenic mouse (The bar represents 200 μm on (a) and 100 μm on (b), (c), (d) fields.)

tal [17], orotic acid [18], clofibrate [19] have mitoinhibitory effects on non-transformed hepatocytes. It is widely accepted that TGF-β1 production is increased in liver cirrhosis which is the most common precancerous condition of human liver [20]. The data are contradictory regarding the TGF-β1 content of human HCC [21–23]. Wolenberg and colleagues [24] found that proliferation of neoplastic hepatocytes *in vivo* cannot be explained by their altered sensitivity to the inhibitory effect of TGF-β1. The modern transgenic technology provides an excellent tool for studying the effect of a molecule in a complex *in vivo* situation. However, this system also failed to give a straight answer as to whether TGF-β1 facilitates or inhibits hepatocarcinogenesis. Factor and colleagues [3] described accelerated hepatocarcinogenesis in TGF-β1 producing mice after diethylnitrosamine (DEN) treatment. Tang and colleagues [25] also reported enhanced liver tumorigenesis in TGF-β1 heterozygous knockout mice, which expressed less TGF-β1 than wild-type animals.

The reason for the failure could be the lack of a simple straightforward answer to this question. The mice used by us carry the same transgene construct as the mice used by Factor and colleagues [3], but the level of TGF-β1 expression was lower in our mice and more prolonged. We also examined whether TGF-β1 had a diverse effect on various carcinogens using three compounds not studied so far in this experimental system. N-OH-AAF is an ultimate carcinogen

which does not require metabolic activation. Thioacetamide results firstly in liver cirrhosis and then later in the development of cancer, this is very similar to the human situation. Aflatoxin B1 is a less potent hepatocarcinogen in mice due to their high glutathione S-transferase activity [26], but nevertheless is able to produce liver tumours in mice [10]. Since TGF-β1 might function as a guardian of genomic stability [27] we were interested in how it might interfere with a compound with low initiating potential. Two such compounds, N-OH-AAF and thioacetamide, resulted in more tumours in the transgenic than in the wild-type mice, but aflatoxin B1 proved to be equally effective in the applied dose and treatment schedule. Since differences between the TGF-β1 producing and control groups were pronounced in the earlier timepoints with N-OH-AAF and thioacetamide, we investigated only the early timepoint after aflatoxin B1 treatment. N-OH-AAF and aflatoxin B1 bind DNA [28, 29] whilst the carcinogenic mechanism of thioacetamide is largely unknown [30], therefore, we can not correlate these divergent results by the specific chemical mechanisms of the applied carcinogens. Our results suggest the connection between TGF-β1 and carcinogenesis is extremely complex and can not be reduced to and explained simply by the anti-proliferative effect of TGF-β1 on normal epithelial tissue. In fact, TGF-β1 may have a bifunctional role in carcinogenesis and depending on conditions may inhibit or facilitate tumorigenesis. Our results infer that TGF-β1 may influence distinctly the potency of various carcinogens.

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Acknowledgements—This work was supported by OTKA T 022737, OTKA 16077, OTKA 29006, and D29111 ETT T 02069/97 and NATO Collaborative Research Grant HTECH. EV 973276.