Virchows Archiv A Pathol Anat (1991) 418:193–199

Virchows Archiv A
Pathological Anatomy and Histopathology

Growth characteristics and metastatic potential of seven intestinal carcinoma lines serially passaged in syngeneic rats

M.S. Martin, F. Martin, M.F. Michel, and A. Hammann

Research Group on Digestive Cancers, INSERM U.252, Faculty of Medicine, 7 Bd J. d'Arc, F-21033 Dijon, France

Received September 3, 1990 / Accepted October 26, 1990

Summary. Transplantable tumour lines were obtained from one duodenal carcinoma induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in the Lewis rat and from six colonic carcinomas induced by 1,2 dimethylhydrazine in BDIX or Fisher rats. The tumours were serially transplanted by the subcutaneous route into homologous syngeneic rats. The seven tumours differ from one another in their histological structure, five of them being well or moderately differentiated adenocarcinomas, and in their capacity to produce neutral or acidic mucins. The seven tumours also differ in their growth rate. The seven lines produced metastases; the metastatic potential and the location of the metastases differed from one line to another. The seven lines kept their original differentiation characteristics through multiple passages, representing several years of transplantation into syngeneic hosts. These tumours represent a useful and diversified model of metastatic intestinal carcinoma, available for basic research and therapeutic trials.

Key words: Experimental colonic adenocarcinoma – Transplantation – Metastases

Introduction

Experimental intestinal carcinomas induced by carcinogenic drugs in rodents are useful models for the study of human colorectal cancer. Primary tumours are obtained by repeated injections of the carcinogen over a long period of time: 6–8 months are necessary to obtain small (50–500 mg), usually multiple colorectal tumours. Transplantable tumour cell lines thus have obvious advantages because they make it possible to rapidly collect large numbers of intestinal tumours or to study therapeutic procedures in a reproducible way. However, transplantable tumours often lose the differentiation characteristics of the original primary, either during the first passage or through the serial passages in the syngeneic host.

We report here long-term experience with seven different lines of intestinal tumours, serially transplanted into syngeneic rats. Through passages for cumulative periods up to 10 years, the tumours kept most of the differentiation characteristics of the primary lesions from which they originated. Furthermore, all the lines were able to produce metastases, a relatively rare characteristic for experimental, well-differentiated, transplantable solid carcinomas.

Offprint requests to: M. Martin

Materials and methods

Three strains of rats were used to produce primary tumours: Lewis, Fisher and BDIX. Colonies of BDIX strain rats are maintained by brother-sister mating in the laboratory. Lewis strain rats were obtained from the Center for Selection of Laboratory Animals, CNRS (Orléans-la-Source) and Fisher strain rats were bought from Iffa-Credo (L'Arbresle, France). Intestinal carcinomas were induced by a weekly 15 mg/kg subcutaneous injection of 1,2 dimethylhydrazine (DMH) (Merck, Darmstadt, FRG) for a period of 28 weeks into BDIX (Martin et al. 1973a) and Fisher rats. Carcinomas of the small intestine were induced in Lewis strain rats by a daily administration of 83 mg *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Schuchardt Laboratories, Munich, FRG) per litre of drinking water during 7 months (Martin et al. 1974).

In most cases samples of the primary tumours weighing approximately 50 mg were transplanted subcutaneously (s.c.) into the anterior thoracic wall of syngeneic animals, as has been previously reported (Martin et al. 1973b). The primary tumour at the origin of the LGA line was inoculated intramuscularly into the first host, but was subsequently transmitted through serial s.c. grafts. The colonic tumour at the origin of the DHE line was inoculated intraperitoneally (i.p.) to the first host and the next five passages were obtained by i.p. inoculation of the haemorrhagic ascitic fluid from peritoneal carcinomatosis. At the sixth passage, a tumour was obtained by s.c. inoculation of a mesenteric solid nodule; the line was then transmitted through serial s.c. inoculations. Tumour growth was estimated by surveying the animals once or twice a week. The tumour graft was considered as established when it was clearly palpated after two successive examinations. The time necessary to obtain tumours of approximately 1 ml volume was also estimated. Animals were usually killed when large tumours ulcerat-

Table 1. Origin of the transplantable intestinal carcinomas

Transplantable line	Primary tumour				
	Strain	Sex	Carcinogen	Location	Histology
DHA	BDIX	M	DMH	Distal colon	WD ADC M+
DHB	BDIX	F	DMH	Distal colon	WD ADC Mo/+
DHC	BDIX	F	DMH	Distal colon	WD ADC M+++
DHD	BDIX	F	DMH	Rectum	WD ADC M+
DHE	BDIX	F	DMH	Distal colon	PD CSR M+++
LGA	Lewis	F	MNNG	Duodenum	WD ADC M++
FHA	Fisher	F	DMH	Caecum	PD CSR M+++

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; ADC, adenocarcinoma; CSR, carcinoma with signet ring cells; M, mucin (PAS) scored from 0 to +++

ed to the skin or when their general state of health deteriorated. However, other animals were killed at an earlier stage of tumour evolution to obtain material, or died during the ether anaesthesia often required to assess tumour volume, survival time was thus not fully representative of the natural evolution of the tumour. Autopsy of the dead or killed animals was performed systematically with complete examination of the peripheral lymph nodes, the thoracic and peritoneal cavities to look for metastases. The transplanted tumour and all the macroscopically suspect organs were systematically submitted to a histological examination. Tumour fragments were fixed in Bouin reagent and embedded in paraffin. Sections 3 μm thick were stained with haematoxylin-phloxin-saffron or with modified trichrome-Masson. Mucinous secretion was studied on sections stained with periodic acid-Schiff's reagent for total glycoproteins or pH 2.5 alcian blue staining for acid glycoproteins.

Samples of transplanted tumours weighing approximately 50 mg were washed in Ham F10 cell culture medium, dipped for 1 h in Ham F10 medium supplemented with 15% fetal bovine serum and 15% dimethylsulphoxide at 4° C, then wiped and stored in cryotubes. The cryotubes were kept overnight at −80° C in a domestic ice box, then transferred into liquid nitrogen. The tumour pieces were rapidly thawed by putting 1 ml Ham F10 medium at 37° C into the cryotube immediately before grafting the piece to syngeneic rats. Multiple fragments of each of the seven tumour graft lines obtained during the early passages were stored in liquid nitrogen to ensure the preservation of the characteristics of the original tumours.

Results

The origin of the seven transplantable intestinal tumours is reported in Table 1. Attempts to establish transplantable carcinoma lines from the original tumours were often unsuccessful, since established transplantable lines were only obtained from 1 out of 5 MNNG-induced small intestine carcinomas in Lewis rats, from 5 out 50 DMH-induced large intestine carcinomas in BDIX rats and from 3 out of 11 DMH-induced colonic carcinomas in Fisher rats. (The FHB and FHC lines were recently obtained and will not be included in this study.)

The success rate of the tumour grafts varied from 52% to 95% according to the line (Table 2). Table 2 also reports, for each of the seven lines, the mean latency time, defined as the time interval between the transplantation and the palpation of a firmly established tumour, and the mean time elapsed before the graft reached an approximate volume of 1 ml. The local growth rate largely differed from slow growing tumours, DHA, DHC or FHA lines to relatively fast growing tumours, LGA, DHB or DHD lines. Tumours with a volume larger than 5–10 ml often adhered to the skin and then ulcerated.

Metastatic potential differed from one cell line to another (Table 3). The rate of metastases found at the autopsy also clearly depended on the time interval between the graft and the killing of the animal. Metastases were found exceptionally when the rats were killed less than 90 days after the tumour graft. Furthermore, they were more often found when the local subcutaneous tumour had been surgically removed, chiefly when recurrence occurred after an intentional or unintentional partial resection. The lung was the most frequently involved organ for almost all the tumour lines. Only DHB and DHD tumour grafts produced metastases to the kidney. Metastases to liver were never observed, either during the first ten passages reported in Table 3, or during the subsequent passages.

Through the first ten passages, the seven transplantable tumour lines kept their own histological characteris-

Table 2. Success rate and local growth characteristics of the seven lines of tumour grafts (passages 2–10)

Line	Success rate	Latency time[a] (days)	Time to reach 1 ml of volume (days)
DHA	121/161 (75%)	53 (24)[b]	110 (53)[b]
DHB	118/166 (71%)	27 (15)	56 (27)
DHC	46/88 (52%)	46 (21)	110 (64)
DHD	124/160 (78%)	24 (30)	44 (31)
DHE	68/114 (60%)	43 (27)	83 (46)
LGA	87/92 (95%)	24 (12)	38 (17)
FHA	110/170 (65%)	55 (25)	113 (50)

[a] Time interval between the tumour graft and the presentation of an established tumour graft
[b] Standard deviation

Table 3. Metastatic potential of the seven tumour graft lines

Line	DHA	DHB	DHC	DHD	DHE	FHA	LGA
Ratio of rats bearing metastases							
—without resection of the graft	5/54	7/19	0/17	1/9	5/26	4/91	0/1
—with resection without recurrence	1/23	9/25	2/15	5/35	1/17	0/2	2/10
—with resection and recurrence	13/36	35/50	6/15	18/28	17/21	1/11	5/25
Time interval (days) between graft and the killing of metastases-bearing rats	249 (65)[a]	168 (37)	239 (32)	159 (78)	175 (52)	258 (141)	130 (26)
Number of rats bearing metastases							
—to lung	17	51	3	24	17	1	6
—to mediastinal lymph nodes	9	16	5	6	9	3	2
—to axillary lymph nodes	0	6	3	3	5	2	1
—to kidney	0	21	0	7	0	0	0
—to diaphragm, pleura, pericardium	6	5	1	1	7	0	1
—to bone	0	2	0	1	0	0	0
—to liver	0	0	0	0	0	0	0
Number of rats bearing at least one metastasis	19/113	51/94	8/47	24/72	23/64	5/104	7/36

Only the data concerning passages 2–10 of each graft line were included in this table. All the animals living less than 90 days after the tumour transplantation were excluded from this study.

[a] Standard error

tics, which were identical to those of the primary intestinal tumours from which they originated (Table 4). There were considerable variations from one line to another in the intestinal differentiation of the tumours (Fig. 1a–g). LGA was a mucus-producing papillary adenocarcinoma. DHA, DHB and DHD tumours were well-differentiated tubular adenocarcinomas differing in their capacity to produce mucus (none in DHB, poor and sparse in DHD, high in DHA). DHC was a moderately differentiated mucus-secreting carcinoma, mixing glandular structures, cords or lobules of cylindrical or cuboidal tumour cells and signet ring cells. DHE and FHA were poorly differentiated mucinous carcinomas with clusters of epithelial cells and isolated signet ring cells scattered in an abundant stroma containing extracellular mucus. Small foci of osteoid metaplasia were occasionally found in the stroma of LGA and FHA tumours. Alcian blue reaction, characteristic of acidic mucin, was strongly positive for DHA, DHC and LGA tumours, weak and variable for DHE and FHA tumours and negative for DHB and DHD tumours.

Table 4. Differentiation characteristics of the seven intestinal lines

Transplantable line	Histological type	Mucin secretion	
		PAS	AB
DHA	WD ADC (T)	+++	+++
DHB	WD ADC (T)	0	0
DHC	MD ADC (T+Tr+SR)	+++	++
DHD	WD ADC (T)	+++	+
DHE	PD SRC (Tr+T+SR)	+++	+
LGA	WD ADC (P)	+++	++
FHA	PD SRC (Tr+SR)	+++	+/−

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; ADC, adenocarcinoma; SRC, signet ring cell carcinoma; T, tubular; TR, trabecular; SR, signet ring cells; P, papillary; PAS, periodic acid-Schiff; AB, alcian blue, pH 2.5 scored from 0 to +++

DHA, DHB, DHC, DHD, DHE, FHA and LGA tumour lines were serially transplanted, 14, 46, 20, 36, 39, 17 and 27 times respectively into syngeneic rats, for cumulative periods of in vivo growth ranging from 4 to 10.6 years. Except for the first two or three passages, which usually grew more slowly than the following ones, early passages had the same growth characteristics and metastatic potential as the late ones. Likewise, there was no major change in the histological type, between early and late passages for most of the seven tumour lines. However, the DHD tumour line changed from faintly PAS-positive to entirely negative for PAS mucus staining. PAS-positive signet ring cells disappeared from the DHE tumour line from the 28th or the 30th passage on.

When rats were grafted with fragments of syngeneic tumours stored in liquid nitrogen, the success rate was similar to that of fresh tumour grafts, ranging from 56% for DHE tumour to 92% for LGA tumour. There was no change in the histology of the transplanted tumour, even when the graft was stored for up to 9 years in liquid nitrogen.

Discussion

Some of the seven transplantable intestinal tumour lines used in this work were described in a previous publication (Martin et al. 1973b). At that time, this was the first report of a transplantable model of intestinal tumours. Since then, several lines of chemically induced colorectal carcinomas transplantable into syngeneic rats (Ward et al. 1973; McCall and Cole 1974; Goto et al. 1975; Steele et al. 1975; Cobb et al. 1987) or mice (Cor-

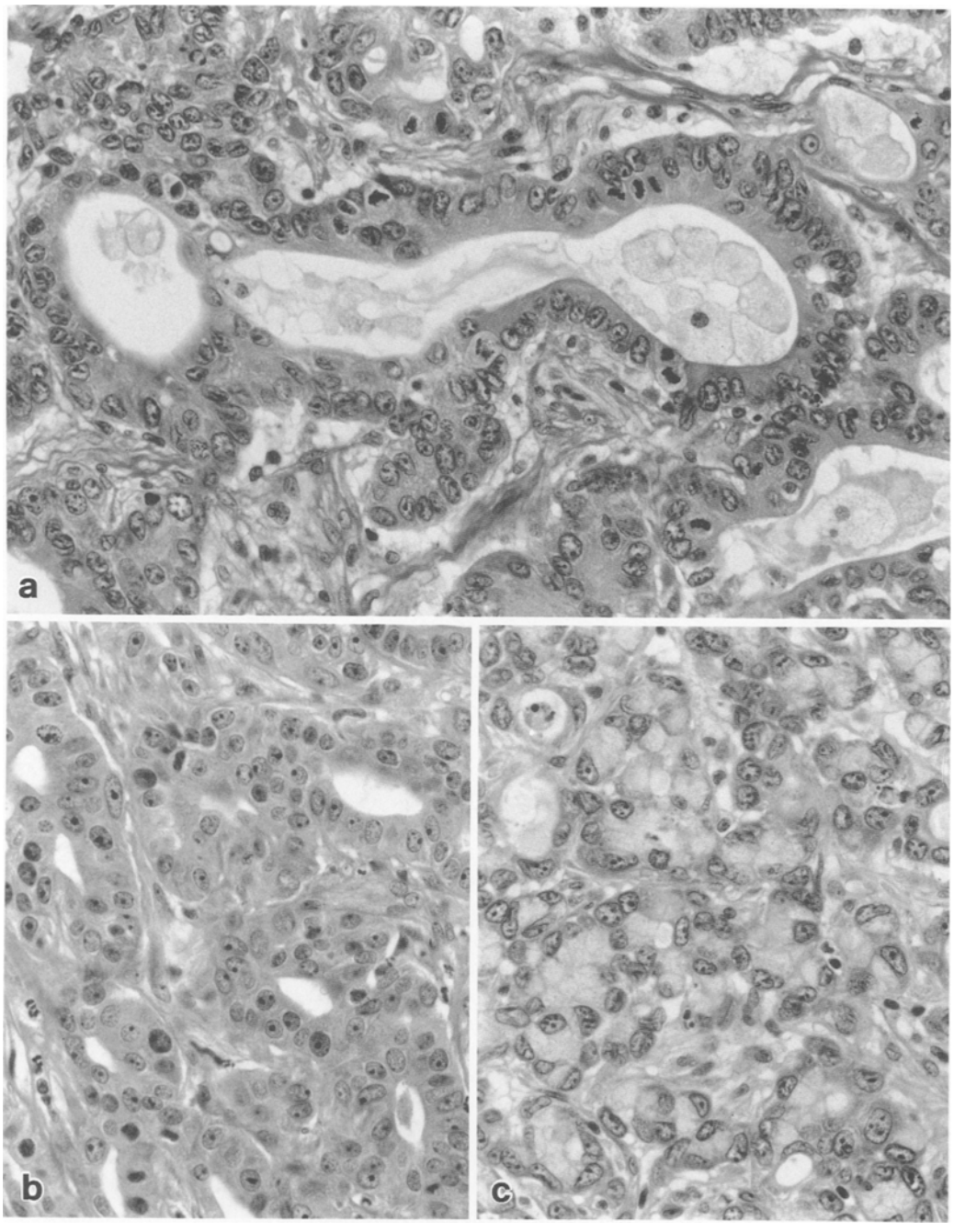

Fig. 1. (a–g). DHA (**a**), DHB (**b**) and DHC (**c**) colonic adenocarcinomas serially transplanted into BDIX rats. Trichrome, ×360. DHD (**d**) and DHE (**e**) colonic adenocarcinomas serially transplanted into BDIX rats. FHA (**f**) and LGA (**g**) are respectively a colonic carcinoma grafted into a Fisher rat and a duodenal adenocarcinoma grafted into a Lewis rat. Trichrome, ×360

bett et al. 1975; Double et al. 1975) have been reported. The murine intestinal tumours obtained by Griswold and Corbett (1975) and by Cowen et al. (1982) were widely used to evaluate the efficiency of chemotherapeutic agents.

As the seven transplantable tumour lines reported here have been serially passaged in syngeneic rats for long periods of time, it seemed interesting to summarize their main characteristics but also their stability throughout multiple serial passages. The lines kept the main features of the primary chemically induced tumours from which they were derived. Their histological patterns varied from one line to another. These histological features mimicked the characteristics of human colorectal tumours, which consist mostly of well or moderately differentiated adenocarcinomas and rarely of signet ring cell carcinomas (Morson and Dawson 1979).

The seven lines exhibited different characteristics in their local outgrowth and in their metastatic potential. Local growth was relatively slow when compared with tumour growth of numerous experimental models, including mouse colonic transplantable lines (Griswold

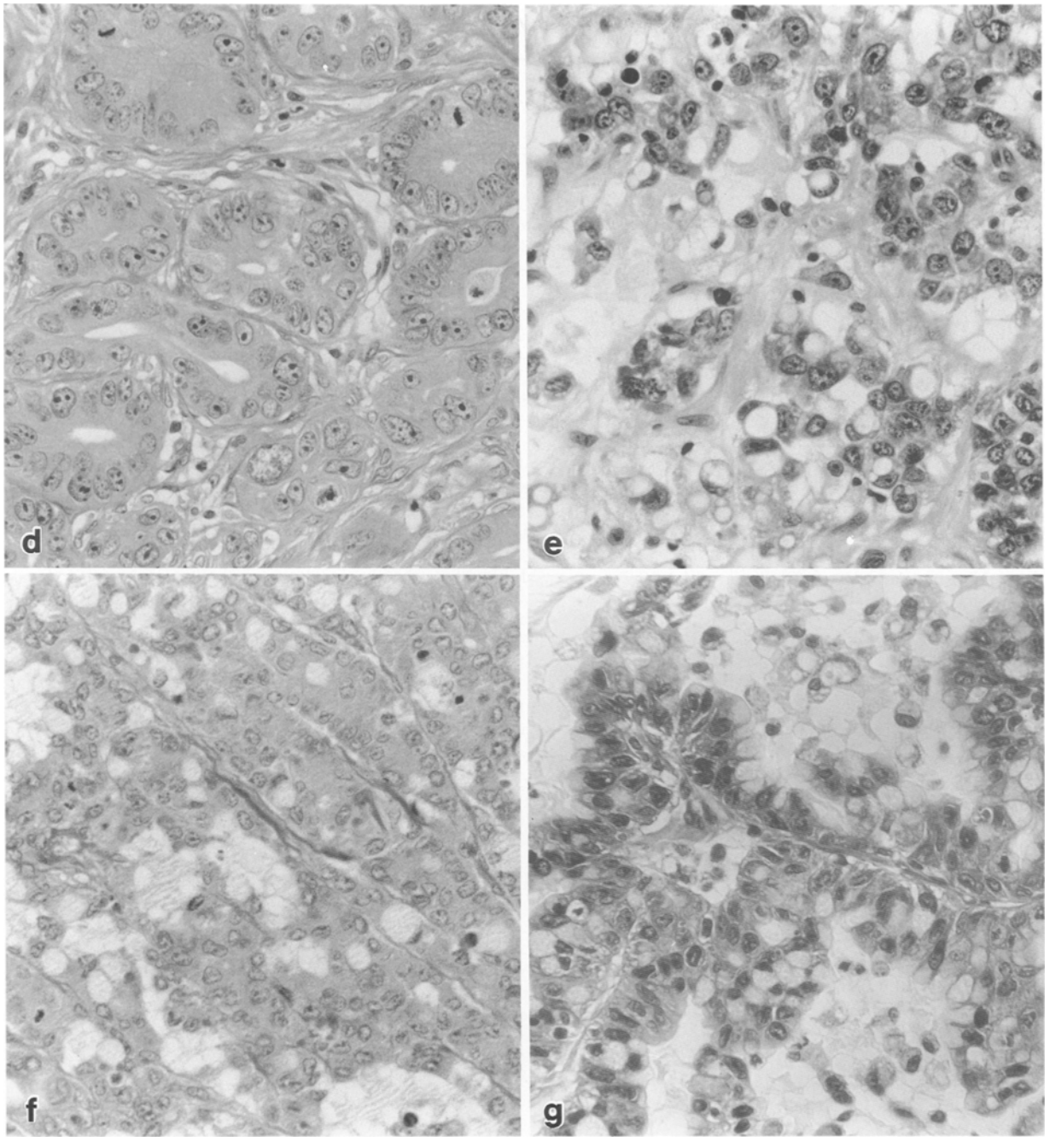

Fig. 1

and Corbett 1975). This slow local growth correlated better with the local evolution of the human colorectal tumours, whose doubling time has been estimated at more than 150 days (Charbit et al. 1971). The local growth was specially slow for DHA, DHC and FHA tumour lines, which reached a volume of 1 ml only 3 or 4 months after transplantation. Spreading to local lymph nodes or distal metastases were observed in all the lines with variations from one line to another. Lymph node invasion or distal metastases were only observed in animals which survived at least 3 months after tumour implantation. When the tumour had a rapid local growth with skin ulceration, as in the LGA line, animals rarely survived 3 months unless the tumour had been partly resected. Repeated partial resections of tumours were the best way to obtain metastases. Metastases could thus been induced in 70% of the rats grafted with DHB line, in 64% with DHD and in 81% with DHE. All the lines provided lung metastases or axillary and mediastinal lymph node invasion, whereas kidney and bone metastases were obtained only with DHB and DHD lines. It is noteworthy that liver metastases were

never observed in these models of transplantable tumours, whereas liver is the most common site of metastases in human colorectal carcinomas. The most likely reason for this discrepancy may be that the venous drainage of the subcutaneous tissues does not pass to the liver, unlike the venous drainage of the gastrointestinal tract. However, the high incidence of pulmonary metastases obtained with the experimental tumours contrasts with the rarity of pulmonary metastases in the human disease. Tumour emboli from the subcutaneous tissue would be expected to lodge in the lung in the rat, whereas venous emboli from human gastrointestinal carcinomas first reach the liver, where metastases are common. Rat liver may also be particularly resistant to metastatic invasion (Malter et al. 1986). However, artificial liver metastases can be induced by intraportal injection of DHD tumour cells (Van der Elst et al. 1986; Solberg et al. 1988; Chauffert et al. 1988) or other transplantable rat colon carcinomas (Ravikumar et al. 1989).

The seven lines kept a relative stability in spite of multiple serial passages and cumulative in vivo growth for up to 10 years. This stability was rather exceptional for differentiated solid tumours. Griswold and Corbett (1975) reported that murine colon cancer grafts had a tendency to dedifferentiation from the first transplantation on. The seven lines have been stored frozen in liquid nitrogen since the first passages. The rate of success obtained by grafting a frozen tumour fragment was almost equal to that obtained when grafting a fresh fragment. This caution allowed us to retard the serial passages and the risk of dedifferentiation.

Cell cultures were established from four of the seven lines, so that large amounts of pure tumour cells were available. These cell cultures were used by ourselves (Martin et al. 1975; Martin et al. 1983; Chauffert et al. 1986; Chauffert et al. 1988) and by others (Van der Elst et al. 1986; Dunnington et al. 1987; Solberg et al. 1988) to study the immunology and the biology of colonic tumours or to evaluate treatments by cytotoxic drugs or modulators of the biological response. Cultured cell lines gave tumours when grafted into syngeneic animals; these tumours were, however, less differentiated than the original serially transplanted tumour lines. Cultured cell lines were obtained from other tumours transplanted in the mouse and in the rat (Borman et al. 1982; Tsuruo et al. 1983; Bresalier et al. 1987).

The transplantable lines reported here represent a good experimental model of human colorectal tumours. They reproduce the different histological patterns of human carcinomas. Like human tumours they grow slowly and they can induce metastases. However, when compared with human tumours, the great difference exists in the fact that they do not induce liver metastases. These tumours are stable when serially grafted in vivo and can be kept frozen in liquid nitrogen for many years. Together with murine transplantable lines, they offer more opportunities to study the biology of colorectal cancer and to experiment with new therapeutic approaches to these tumours.

Acknowledgements. The authors thank Prof. E. Justrabo for her valuable advice and Mrs. C. Perreau-Philippe for her help in revising this manuscript.

References

Borman LS, Swartzendruber DC, Littefield LG (1982) Establishment of two parental cell lines and three clonal cell strains from a rat colonic carcinoma. Cancer Res 42:5074–5083

Bresalier RS, Hujanen ES, Raper SE, Roll FJ, Itzkowitz SH, Martin GR, Kim SY (1987) An animal model for colon cancer metastasis: establishment and characterization of murine cell lines with enhanced liver metastasizing ability. Cancer Res 47:1398–1406

Charbit A, Malaise EP, Tubiana M (1971) Relation between pathological nature and growth of human tumours. Eur J Cancer 7:577–582

Chauffert B, Martin M, Hammann A, Michel MF, Martin F (1986) Amiodarone-induced enhancement of doxorubicin and 4′-deoxydoxorubicin cytotoxicity to rat colon cancer cells in vitro and in vivo. Cancer Res 46:825–830

Chauffert B, Shimizu T, Caignard A, Hammann A, Genne P, Pelletier H, Martin MS (1988) Use of a specific monoclonal antibody for studying the liver metastatic invasion of a rat colon cancer. In Vivo 2:301–306

Cobb RA, Gartell PC, Steer HW, Shurbier A, Garrod MA (1987) Transplantable colonic adenocarcinomas in rats. Dis Colon Rectum 30:255–262

Corbett TH, Griswold DP, Roberts BJ Jr, Peckham JC, Schabel FM (1975) Tumor induction relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. Cancer Res 35:2434–2439

Cowen DM, Siegerstetter J, Janick AC, Double JA (1982) The assessment of response of murine transplantable colon tumors to combination chemotherapy. J Cancer Res Clin Oncol 103:119–126

Double JA, Ball CR, Cowen PN (1975) Transplantation of adenocarcinomas of the colon in mice. J Natl Cancer Inst 54:271–275

Dunnington DJ, Buscarino C, Gennaro D, Greig R, Poste G (1987) Characterization of an animal model of metastatic colon carcinoma. Int J Cancer 39:248–254

Goto K, Kurokawa Y, Hayashi J, Sato H (1975) Transplantable adenocarcinomas from colorectal tumors induced by infusion of N-methyl-N′-nitro-N-nitrosoguanidine in ACI/N rats. Gann 66:89–93

Griswold DVM, Corbett THA (1975) Colon tumor model for anticancer agent evaluation. Cancer 36:2441–2444

Malter M, Friedrich EA, Süss R (1986) Liver as tumor cell killing organ: Küpffer cells and natural killers. Cancer Res 46:3055–3060

Martin MS, Martin F, Michiels R, Bastien H, Justrabo E, Bordes M, Viry B (1973a) An experimental model for cancer of the colon and rectum: intestinal carcinoma induced in rat by 1,2 dimethylhydrazine. Digestion 8:22–34

Martin MS, Bastien H, Martin F, Michiels R, Martin MR, Justrabo E (1973b) Transplantation of intestinal carcinoma in inbred rats. Biomedicine 12:555–558

Martin MS, Martin F, Justrabo E, Michiels R, Bastien H, Knobel S (1974) Susceptibility of inbred rats to gastric and duodenal carcinomas induced by N-methyl-N′-nitro-N-nitrosoguanidine. J Natl Cancer Inst 53:837–840

Martin F, Knobel S, Martin MS, Bordes M (1975) A carcinofetal antigen located on the membrane of cells from intestinal carcinoma in culture. Cancer Res 35:333–336

Martin F, Caignard A, Jeannin JF, Leclerc A, Martin MS (1983)

Selection by trypsin of two sublines of rat colon cancer cells forming progressive or regressive tumors. Int J Cancer 32:623–627

McCall DC, Cole JN (1974) Transplantation of chemically induced adenocarcinomas of the colon in an inbred strain of rats. Cancer 4:1021–1026

Morson BC, Dawson IMP (1979) Adenocarcinoma and other malignant epithelial tumours. In: Morson BC, Dawson IMP (eds) Gastrointestinal pathology. Blackwell, Oxford, pp 648–680

Ravikumar TS, D'Emilia J, Cocchiaro C, Wolf B, King V, Steele G (1989) Experimental liver metastases. Arch Surg 124:49–54

Solberg E, Gjoen T, Seljelid R, Kolset SO (1988) Colonization of the rat liver by syngeneic tumor cells. An experimental approach by in vivo and in situ studies. Virchows Arch [B] 55:111–116

Steele G, Sjögren HO, Price MR (1975) Tumor associated and embryonic antigens in soluble fractions of a chemically induced rat colon carcinoma. Int J Cancer 16:33–51

Tsuruo T, Yamori T, Naganuma K, Tsukagoshi S, Sakurai Y (1983) Characterization of metastatic clones derived from a metastatic variant of mouse colon adenocarcinoma 26. Cancer Res 43:5437–5442

Van der Elst J, Greve J de, Beerst F, Neve W de, Storme G, Willems G (1986) Quantitative study of liver metastases from colon cancer in rats after treatment with cyclosporine A. J Natl Cancer Inst 77:227–231

Ward JM, Yamamoto RS, Weisburger JH, Benjamin T (1973) Transplantation of chemically induced metastatic mucinous adenocarcinomas of the jejunum and colon in rats. J Natl Cancer Inst 51:1997–1999