



CHARACTERIZATION OF AN ANIMAL MODEL OF METASTATIC COLON CARCINOMA

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Although numerous animal tumor models have been used to study colon carcinoma, few display metastatic properties. We have characterized an animal tumor model that has 3 properties essential for the study of metastasis of colon carcinoma cells: epithelial cell origin; a reproducible pattern of metastatic behavior and the ability to be propagated both *in vitro* and *in vivo* to facilitate identification of biochemical correlates of metastasis. The K12/TR cell line was derived from a transplantable colon carcinoma induced by dimethylhydrazine in the BD-1X rat strain. Transmission electron microscopy of K12/TR cells demonstrated junctional complexes, desmosomes and surface microvilli characteristic of gastrointestinal epithelial cells. The epithelial cell origin of K12/TR was confirmed by demonstrating the presence of keratin, a marker of epithelial cells, but not vimentin, a constituent of mesenchymal cells. Secretion of CEA and Ca19-9 antigens by K12/TR cells *in vitro* was below the sensitivity of the assays (1 ng/ml and 6 U/ml respectively). K12/TR cells produced tumors following s.c. injection into syngeneic BD-1X rats, allogeneic RNU/rnuDF rats and xenogeneic CRL:nu/nuBR mice. Macroscopic lung metastases were observed in animals from all 3 groups. Distal lymph node metastases were more frequent in BD-1X rats than in nude rats or mice. The histological appearances of all tumors and metastases were similar, showing a moderate to poorly differentiated glandular carcinoma. Intrasplenic injections of K12/TR cells in nude mice resulted in liver colonization. Preferential growth of tumor cells at sites of trauma was also observed. The results show that the K12/TR system can be used as a model to study metastasis of colon carcinoma cells and may find utility in the testing of chemotherapeutic agents against metastatic lesions.

A variety of experimental colon tumors have been studied as models for human colorectal cancer and for testing chemotherapeutic drugs. These include rodent tumors induced by a structurally diverse panel of chemical carcinogens (Weisburger *et al.*, 1983), transplantable tumor lines derived from such lesions (Corbett *et al.*, 1975), and xenografts of human colon carcinoma in nude mice (Dexter *et al.*, 1979) or rats (Drewnko *et al.*, 1984). However, in contrast to clinical disease in man, few of these experimental models display reproducible metastatic properties. Indeed, human tumor xenografts in nude mice are notable for their lack of metastatic behavior (Sharkey *et al.*, 1979). However, Kozlowski *et al.* (1984) reported limited production of pulmonary metastases after s.c. injection of the human colon carcinoma cell line HT29 into nude mice and more extensive dissemination after intrasplenic injection. More recently, a model for colon cancer metastasis using intrahepatic administration of a transplantable human colon carcinoma in immunocompetent Syrian hamsters was described (Sharkey *et al.*, 1986).

We wished to develop a model system of metastatic colon carcinoma suitable for the evaluation of therapeutic modalities in immunocompetent, syngeneic hosts as well as immunodeprived animals. Preliminary clinical studies using vaccines prepared from autologous tumor cells have reported significant retardation of disease progression in colorectal cancer patients (Hoover *et al.*, 1985), and a reduced rate of recurrence of chemically-induced rat colon carcinomas (Ross *et al.*, 1984). A syngeneic system is particularly useful for immunotherapeutic maneuvers of this kind and also for the routine testing of antineoplastic agents. Two murine colon tumor cell

lines have been reported to metastasize in syngeneic hosts (Tsuruo *et al.*, 1983; Thombre *et al.*, 1984). However, one of these lines, P26, is apparently not of epithelial origin (Tsuruo *et al.*, 1983), and the other requires technically demanding intracecal administration to generate liver metastases, a procedure that does not lend itself readily to screening programs which require a large volume throughput of test materials. Thus there is a clear need for a well-characterized syngeneic model of colon carcinoma metastasis.

Here we report the detailed characterization of the metastatic properties of a rat colon carcinoma cell line designated K12/TR. This cell line was derived by Martin *et al.* (1983) from a transplantable colon carcinoma induced with 1,2 dimethylhydrazine in the syngeneic BD-1X rat strain (Martin *et al.*, 1973). The parental cell strain DHD/K-TR, from which the K12/TR line was obtained by cloning, was shown to metastasize in syngeneic hosts and to display an epithelial morphology (Martin *et al.*, 1983) and appears to fulfil the criteria described above for a suitable animal model system. The present study describes the metastatic properties of the K12/TR cell line in syngeneic, allogeneic and xenogeneic hosts and an initial phenotypic characterization of these cells.

MATERIAL AND METHODS

Animals

Female BD-1X rats were obtained from a breeding colony at NCI, Bethesda, MD and were housed in laminar flow racks. Female nude rats (RNU/rnuDF) were supplied by Harlan Sprague-Dawley (Indianapolis, IN), while female nude mice (Crl:NU/NU) were obtained from Charles River, Wilmington, MA. Nude rats and mice were housed in separate facilities. Sera from representative nude rats were tested at 6-monthly intervals for antibodies to rat coronavirus, sialodacryoadenitis virus, Toolan's H-1 virus, Kilham rat virus, pneumonia virus of mice, Sendai virus and *Mycoplasma pulmonis*. Sera from nude mice were similarly tested for antibodies to Sendai virus, pneumonia virus of mice, minute virus of mice, Theiler mouse encephalomyelitis virus, type 3 reovirus, mouse hepatitis virus, ectromelia, lymphocytic choriomeningitis virus and *Mycoplasma pulmonis*. Animals were allowed free access to food and water.

Cell cultures

The K12/TR line was a gift from Dr. F. Martin (Dijon, France). Cells were cultured as monolayers in DMEM, sup-

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Abbreviations: PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetate; DMEM, Dulbecco's modified Eagle's medium; CEA, carcinoembryonic antigen; Ca19-9, carbohydrate antigen 19-9; BSA, bovine serum albumin; s.c., subcutaneous.

plemented with 10% fetal bovine serum. Cells were subcultured by dispersal with 0.125% trypsin in 0.125% EDTA after prior exposure for 5 min to 0.25% EDTA solution and replating at a 1:10 split. Cultures were screened at regular intervals for the presence of adventitious agents. Cultures were not infected with Mycoplasma (assayed by staining with Hoechst 33258 or by broth culture) and were free from the following viruses as assayed by a murine antibody production (MAP) test (Microbiological Associates, Bethesda, MD): Sendai virus; mouse hepatitis virus; pneumonia virus of mice; type 3 reovirus; Theiler mouse encephalomyelitis virus; K virus; ectromelia; minute virus of mice; polyoma; mouse adenovirus; and lymphocytic choriomeningitis virus. The rat origin of the K12/TR cultures was confirmed by isoenzyme analysis using the Corning Authentikit System (Corning, NY).

Expression of intermediate filaments and colon-associated markers

Immunophenotyping of cell populations was carried out using the antisera to keratin, a marker of epithelial cells (Moll *et al.*, 1982), vimentin, a marker of nonepithelial cells (Franke *et al.*, 1979), carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (Ca 19-9), markers associated with, but not specific to, colon cells (Laurence *et al.*, 1972; Koprowski *et al.*, 1981). Expression of keratin or vimentin was assessed by indirect immunofluorescence. Cells grown on glass slides (Lab-Tek, Miles Scientific, Naperville, IL), were fixed for 5 min in methanol (-20°C) and incubated with a 1/20 dilution of monoclonal antiserum to keratin or vimentin (Labsystems, Chicago, IL) in distilled water containing 0.1% BSA. Slides were washed with PBS (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, pH 7.2) and stained for 30 min with fluorescein-conjugated goat anti-mouse serum (Cappel, Cochranville, PA). The cells were then washed with PBS, mounted in glycerol and observed in a fluorescence microscope. CEA and Ca 19-9 were assayed using commercially available radioimmunoassay kits (Abbott, N. Chicago, IL, and SmithKline Bioscience, respectively) to measure the antigen content of cell-free medium collected from 10-day cultures of K12/TR cells.

Electron microscopy

Confluent monolayers of K12/TR cells were washed with PBS and fixed with 2% glutaraldehyde, 1 mM CaCl_2 in 0.1 M cacodylate buffer, pH 7.4. Cells were scraped from the plastic substratum using a rubber spatula and centrifuged at 200 g for 5 min. Samples of non-necrotic portions of tumors induced by s.c. injections of K12/TR cells in syngeneic rats were fixed in the same solution. Both cell pellets and tumor samples were processed for transmission electron microscopy using standard methods and examined in a Jeol CX100 at 80kV.

Metastatic properties

Monolayer cultures of K12/TR cells were grown to near confluence in 175 cm^2 flasks, washed twice with PBS and suspended by incubation at 37°C with 0.25% EDTA for 5 min followed by trypsinization as above. Aliquots of the cell suspensions were stained with Trypan blue and counted with a hemocytometer. The cells were washed in DMEM plus 10% fetal bovine serum to inactivate trypsin, resuspended at 10^7 viable cells/ml PBS and injected into animals. Rats (syngeneic BD-1X and nude) received 0.1 ml cell suspension injected at either of 2 s.c. sites (anteriolateral thoracic and flank) or via the intraperitoneal or intrasplenic (Kozlowski *et al.*, 1984) routes.

Nude mice were inoculated by similar routes of administration, but groups of animals were also injected in the footpad (1×10^6 cells in 0.05 ml PBS). Footpad tumors were amputated under Metofane (Pitman-Moore, NJ) anesthesia 32 days

post-injection on reaching a size of approximately 1.5 cm. Groups of 5 nude mice were injected via a lateral tail vein with 10^3 , 5×10^4 or 10^6 K12/TR cells in 0.05 ml PBS.

A further group of 5 nude mice were anesthetized with Metofane and puncture wounds made with an 18-gauge needle through the left abdominal wall. A control group of 5 mice received puncture wounds through the skin but without penetration of the abdominal wall. Mice in both groups were then injected with 10^6 K12/TR cells in 0.5 ml PBS in the contralateral side of the abdomen, using a 25-gauge needle. Animals were examined for evidence of tumor growth at the injury sites 14 days post-injection.

RESULTS

In vitro growth characteristics of K12/TR cells

K12/TR cells grew as tightly packed colonies in monolayer cultures and individual cells displayed a cuboidal morphology (Fig. 1a). Spontaneous dome formation, a characteristic of vectorial fluid transport by epithelial cells (McGrath, 1974), was observed in confluent cultures (Fig. 1a). Population doubling time measured by sequential cell counts was approximately 28 hr (data not shown). K12/TR cells stained strongly with a monoclonal antiserum to porcine kidney keratin (Fig. 1b) but did not stain with antiserum to vimentin (Table I). Control cultures of B16 murine melanoma cells or human colon fibroblast (CCD18) cells stained strongly with antiserum to vimentin but failed to stain for keratin (not shown).

Media harvested from 10-day K12/TR cultures contained no detectable CEA or Ca 19-9 as measured by radioimmunoassay (Table I). The assay sensitivity limits were 1 ng/ml and 6U/ml for CEA and Ca 19-9, respectively. Control samples of the human colon carcinoma cell line "LOVO" were positive for both CEA and Ca 19-9 in the same assay.

Ultrastructural analysis

Both cultured K12/TR cells and tumors from syngeneic rats showed ultrastructural features characteristic of epithelial cells. Surface microvilli were present, junctional complexes were observed between adjacent cells, and occasional poorly-formed desmosomes were also seen (Fig. 1c).

Metastatic properties of K12/TR cells in nude mice

Tumors occurred in all mice inoculated with K12/TR cells, regardless of the route of administration. Tumors at s.c. sites were palpable 20 days post-injection. With the exception of 2 animals whose tumors regressed completely, all tumors grew progressively. Tumor-bearing animals developed cachexia and were killed when moribund. Multiple lung metastases were seen in 8 of 9 mice with s.c. tumors at the anteriolateral thoracic site, 4 of 4 mice with tumors in the flank and 6 of 6 mice with footpad tumors (Table II). Multiple lung metastases were also seen in 2 mice whose footpad tumors were not amputated. Nude mice receiving i.p. injections of K12/TR cells developed ascites, and extensive colonization of the body wall, diaphragm and fatty tissues was noted at autopsy. In mice given intrasplenic injections, tumors were seen at the site of s.c. incision as well as in the spleen. The s.c. tumors were much larger (2-3 cm diameter) than the splenic tumors (0.5-1 cm) and highly invasive. Liver colonization was noted in 5 of 6 of these animals, but lung metastases were not seen (Table II). Lymph-node metastases were rarely observed in nude mice, at either a gross or a microscopic level (Table II). One mouse bearing an intrafootpad tumor had a large (0.4 cm) tumor in the para-aortic lymph node, but other mice in the same group were devoid of lymph-node lesions. All mice injected i.v. with either 10^6 or 5×10^4 K12/TR cells exhibited lung tumors (Table II). It was not possible to count individual nodules due to their density. Animals became moribund 15

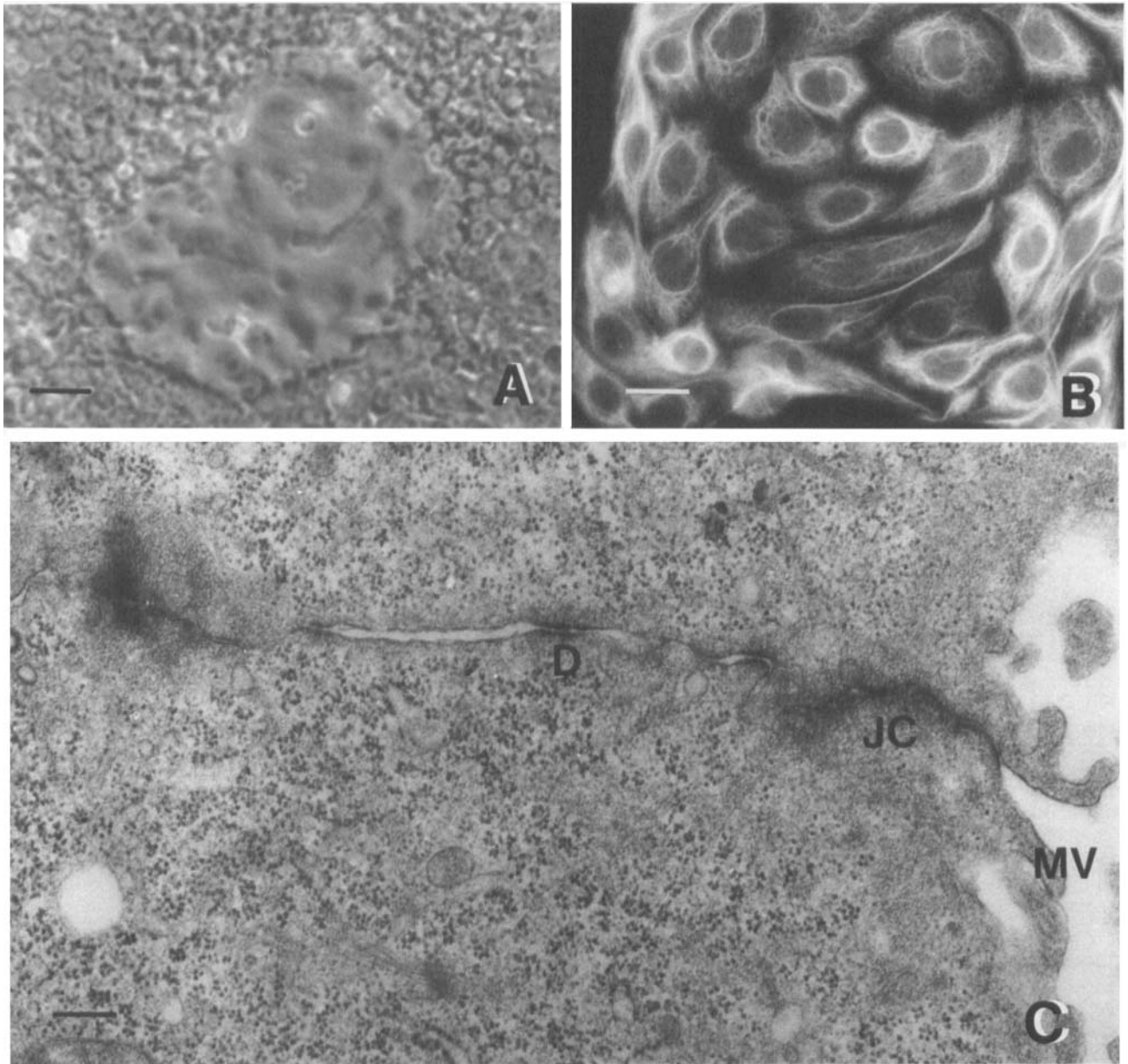


FIGURE 1 – Morphological characterization of K12/TR Cells *in vitro*. (a) Phase-contrast photomicrograph of K12/TR cells in culture showing dome formation. (b) Immunofluorescence staining of K12/TR cells with antiserum to keratin showing perinuclear fibrillar fluorescence. (c) Transmission electron micrograph of cultured K12/TR cells showing sparse microvilli (MV), desmosome (D) and junctional complex (JC).

days post-injection in the high-dose group and within 58-81 days of receiving 5×10^4 K12/TR cells. A dose of 10^3 cells i.v. failed to induce lung colonies 92 days post-injection, except in the case of one animal in which a single 3-mm pulmonary nodule was observed (Table II).

The growth of K12/TR tumors at incision sites of mice injected via the intrasplenic route suggested that trauma might enhance tumor growth. To examine this possibility, nude mice were wounded at a contralateral site on the abdominal wall before i.p. injection of K12/TR cells. In animals with wounds that penetrated the body wall, tumors were seen at the injury sites 14 days post-trauma (Fig. 2a,b). In contrast, tumors were

not seen in puncture wounds of limited depth that did not penetrate the body wall. In animals with the penetrating wounds colonization of injury sites occurred more rapidly and the tumors attained a larger size than the diffuse growth observed in the body wall of control animals.

Metastatic properties of K12/TR cells in nude rats

The metastatic behavior of K12/TR cells observed in nude mice was also seen in nude rats. Multiple lung metastases were seen in animals bearing s.c. tumors, but lymph-node metastases were seen in only 1 of 10 rats with anteriolateral thoracic tumors and in 5 of 5 animals with tumors in the flank. Lymph-

node metastases from tumors growing in the flank were observed only in the ipsilateral nodes (Table III). Tumor growth at the incision sites was seen in nude rats receiving intrasplenic injections of K12/TR cells and these animals also developed tumor deposits in the spleen (Table III). Colonization of the body wall, diaphragm and fatty tissue and ascites production

occurred in rats given i.p. injections of K12/TR cells (Table III). Hepatic surface nodules were present in regions in contact with diaphragm tumors in both the i.p. and intrasplenic groups, but colonization of the liver parenchyma was not observed. Lung metastases were not detected in rats receiving i.p. injections of K12/TR cells, but lung nodules occurred in 4 of 5 rats receiving intrasplenic injections (Table III).

Metastatic properties of K12/TR cells in syngeneic rats

Syngeneic BD-1X rats inoculated s.c. with K12/TR cells showed extensive lung metastases at autopsy (Fig. 2c, Table IV). In addition, animals with anteriolateral thoracic tumors had large metastases in the contralateral axillary lymph nodes (Fig. 2c, Table IV). Intraperitoneal injection of K12/TR cells produced ascites and colonization of the body wall, diaphragm and fatty tissue (Table IV). Occasional surface liver nodules were seen in these animals in areas of contact with diaphragm tumors. Intrasplenic injection of K12/TR cells produced tumors at the incision site and in the spleen, but no lung or liver metastases were observed (Table IV).

TABLE I - EXPRESSION OF INTERMEDIATE FILAMENTS AND COLON ASSOCIATED MARKERS BY K12/TR CELLS *IN VITRO*

Marker	Amount detected ¹	Assay
Keratin	+++	Immunofluorescence
Vimentin	Not detected	Immunofluorescence
CEA ²	< 1 ng/ml	Radioimmunoassay
Ca 19-9 ²	< 6 U/ml	Radioimmunoassay

¹Based on fluorescence intensity: + = weak; ++ = moderate; +++ = intense fluorescence. Control cultures of human colon fibroblasts and mouse B16 melanoma cells stained in parallel were positive for vimentin but not keratin (not shown). ²CEA and Ca 19-9 antigens in 10 day conditioned medium were measured using radioimmunoassay. The detection limits of the assays were 1ng/ml CEA and 6U/ml Ca 19-9. Control samples of the human colon carcinoma cell line "LOVO" were positive for both CEA and Ca 19-9 in the same assay.

TABLE II - METASTATIC PROPERTIES OF K12/TR CELLS IN NUDE MICE

Site ¹	Dose	Tumor incidence ¹	Incidence of metastases ¹			Days (range) to autopsy
			Lung	Lymph node ²	Liver	
sc (alt)	10 ⁶	9/10 ³	8/10	0/10	0/10	72 (43-88)
sc (flank)	10 ⁶	4/5 ³	4/5	2/5	0/5	86 (74-92)
ifp	10 ⁶	6/6	6/6	1/6	0/6	57 ⁴
ip	10 ⁶	5/5 ⁵	0/5	0/5	2/5 ⁶	57 ⁴
is	10 ⁶	6/6 ⁷	0/6	0/6	5/6	23 (15-29)
iv	10 ⁶	N/A	5/5	0/5	0/5	17 (15-23)
iv	5 × 10 ⁴	N/A	4/4	0/4	0/4	64 (58-81)
iv	10 ³	N/A	1/4	0/4	0/4	92 ⁴

¹alt = anteriolateral thoracic; sc = subcutaneous; ifp = intrafootpad; ip = intraperitoneal; is = intrasplenic; iv = intravenous. Figures are the incidence of lesion/number of animals injected; N/A = not applicable. ²Metastases were seen in the ipsilateral axillary and para-aortic nodes in mice in the flank and ifp groups respectively. ³One tumor regressed. ⁴All animals killed at this time. ⁵Ascites formation and tumor colonization of body wall, fatty tissue and diaphragm. ⁶Surface liver nodules were found at areas in direct contact with diaphragm tumor deposits. ⁷Tumors detected at the incision site and in the spleen.

TABLE III - METASTATIC PROPERTIES OF K12/TR CELLS IN NUDE RATS

Site ¹	Dose	Tumor incidence ¹	Incidence of metastases ¹			Days (range) to autopsy
			Lung	Lymph node ²	Liver	
sc (alt)	2 × 10 ⁶	10/10	10/10	1/10	0/10	81 (67-98)
sc (flank)	10 ⁶	5/5	4/5	5/5	0/5	109 ³
ip	10 ⁶	6/6 ⁴	0/6	0/6	2/6 ⁵	27 ³
is	10 ⁶	5/5 ⁶	4/5	0/5	2/5 ⁵	79 (76-91)

¹alt = anteriolateral thoracic; sc = subcutaneous; ifp = intrafootpad; ip = intraperitoneal; is = intrasplenic. Figures are the incidence of lesion/number of animals injected. ²Contralateral and ipsilateral axillary nodes for the anteriolateral thoracic and flank groups respectively. ³All animals killed at this time. ⁴Ascites formation and tumor colonization of body wall, fatty tissue and diaphragm. ⁵Liver nodules found at areas in direct contact with diaphragm deposits. ⁶Tumors detected at the incision site and in the spleen.

TABLE IV - METASTATIC PROPERTIES OF K12/TR CELLS IN SYNGENEIC BD-1X RATS

Site ¹	Dose	Tumor incidence ¹	Incidence of metastases ¹			Days (range) to autopsy
			Lung	Lymph node ²	Liver	
sc (alt)	2 × 10 ⁶	10/10	10/10	10/10	0/10	100 (67-118)
sc (flank)	10 ⁶	5/5	5/5	5/5	0/5	121 (111-124)
ip	10 ⁶	6/6 ³	0/6	0/6	5/6 ⁴	41 ⁵
is	10 ⁶	5/5 ⁶	0/5	0/5	0/5	63 ⁵

¹alt = anteriolateral thoracic; sc = subcutaneous; ifp = intrafootpad; ip = intraperitoneal; is = intrasplenic. Figures are the incidence of lesion/number of animals injected. ²Contralateral axillary node. ³Ascites formation and tumor colonization of body wall, fatty tissue and diaphragm. ⁴Liver nodules found at areas in direct contact with diaphragm deposits. ⁵All animals killed at this time. ⁶Tumors detected at the incision site and in the spleen.

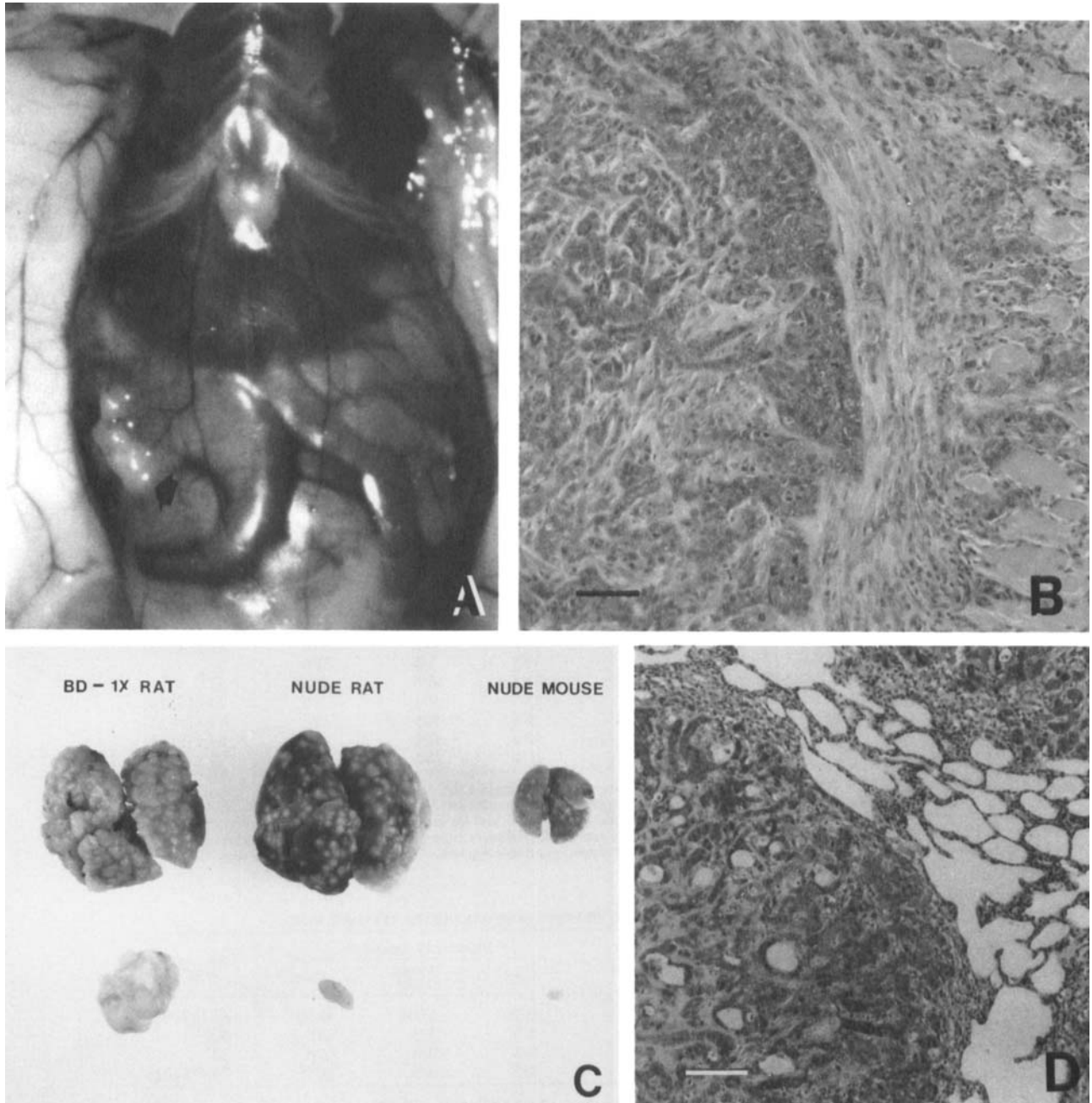


FIGURE 2 - Growth characteristics of K12/TR Cells *in vivo*. (a) Preferential growth of K12/TR cells at the injury site (arrow) in a nude mouse, following i.p. injection of cells. (b) Histological section of the tumor depicted in (a), showing moderately differentiated carcinoma, stromal proliferation and invasion of tumor cells between muscle fibrils. Hematoxylin and eosin. (c) Lungs (top) and contralateral axillary lymph nodes (bottom) from a BD-1X rat, nude rat and nude mouse. Multiple lung metastases are seen in all 3 hosts, and a large lymph-node metastasis is present in the BD-1X rat, but nodes from the nude rat and mouse appear free from gross or microscopic tumor. (d) Histological section of lung metastases, in a BD-1X rat showing moderately differentiated carcinomatous nodules. Hematoxylin and eosin.

Histologic properties of tumor deposits and metastases

The histology of tumors induced at different sites in different hosts was similar. The tumor appeared as a moderately- to poorly-differentiated carcinoma, with extensive infiltrative invasion of host tissues (Fig. 2*b,d*).

DISCUSSION

The purpose of the present study was to establish an animal model for metastatic colon carcinoma. To mimic the human

disease in any reasonable manner, a tumor model must, at least, use colon-derived epithelial cells with defined and reproducible metastatic properties. Although an extensive panel of cell lines have now been derived from human colon carcinoma, their metastatic capacities in nude mice are extremely poor. Furthermore, *in vivo* studies using human tumor xenografts are of necessity limited to immunosuppressed hosts. For many experimental purposes this may be acceptable. However, in attempting to devise models that can be used to evaluate biological response modifiers for their potential in

augmenting host responses to tumors, this is a serious disadvantage. Recent clinical studies suggesting that immunotherapy of colorectal cancer patients may produce significant improvements in short-term prognosis (Hoover *et al.*, 1985) reinforce the need for tumor models for metastatic colon carcinoma in immunocompetent hosts to enable optimum conditions for immunoenhancement to be defined.

Ultrastructural and immunocytochemical findings presented in this study demonstrate that K12/TR cells are of epithelial origin. Unlike many mammary epithelial cells which lose their morphological characteristics during serial cultivation (Bennett *et al.*, 1978; Dulbecco *et al.*, 1981), the epithelial phenotype of K12/TR cells appears to be stable both *in vitro* and *in vivo*. The ability of K12/TR cells to form domes and junctional complexes illustrates the maintenance of epithelial cell polarity needed for vectorial fluid transport. The K12/TR cells do not secrete detectable amounts of CEA or Ca 19-9. Modified forms of these markers may be produced but may not express epitopes recognized by the antisera used in the present assays.

The anatomic patterns of metastasis of K12/TR cells are relatively similar in syngeneic, allogeneic and xenogeneic animals, with the exception of the higher frequency of distal lymph-node metastases in syngeneic rats. The incidence of lymph-node metastases was influenced by the site of primary tumor and by the host species/strain. Dissemination to proximal nodes from an s.c. tumor in the flank was seen in nude rats and at a lower frequency in nude mice. Metastases to contralateral lymph nodes were not observed in nude mice and were present in only one nude rat. Presumably, fewer cells reach the more distal contralateral node and, as observed in other tumors, the incidence of metastases may be determined by a balance between the number of cells reaching the node and the ability of host defenses to eliminate them (Carr, 1983). The K12/TR cell line is known to be immunogenic and its growth in syngeneic animals can be prevented by adoptive transfer of lymphocytes from animals injected with a related cell line, K12/TS (Caignard *et al.*, 1985). It is thus possible that functional elements of the immune system (Gorelik *et al.*, 1982) may limit growth of K12/TR cells in the lymph nodes of nude rats and mice. K12/TR cells were capable of colonizing the lungs of nude mice when introduced directly into the circulation. The time required for the cells to induce morbidity via this route was inversely related to the cell dose. In addition to the similar patterns of metastasis seen in the various host animals in the present study, the parental DHD/K. TR cell line from which K12/TR was derived was shown previously to metastasize to the lungs and lymph nodes of syngeneic BD-1X rats (Martin *et al.*, 1983). Thus, this cell lineage appears to exhibit reproducible metastatic properties in the syngeneic host.

In humans, the liver is the most common site of colon carcinoma metastasis (Sugarbaker *et al.*, 1982). Tumors induced by subcutaneous injection of K12/TR cells failed to form detectable liver metastases. However, liver colonies were

observed following intrasplenic injection of K12/TR cells in nude mice, but their pathogenesis is ambiguous because the cells form massive tumors at the site of incision. Thus, it is not known whether the liver colonies arise directly from the spleen tumors and/or from those at the incision site. No liver colonies were seen in nude mice following i.p. injection of K12/TR cells, except in areas of the liver in direct contact with diaphragm tumor deposits. These colonies were restricted to the surface of the liver as opposed to the parenchymal replacement seen following intrasplenic administration of cells. Thus it is likely that dissemination from the spleen to the liver occurs via the circulation rather than by seeding of the peritoneal cavity. Nonetheless, as reported previously (Kozlowski *et al.*, 1984; Lafreniere *et al.*, 1986), intrasplenic injection of tumor cells in nude mice appears to be a useful means of producing liver tumors for testing therapeutic or diagnostic modalities. Formation of gross or microscopic lung metastases following intrasplenic injection of K12/TR cells was only seen in nude rats. Nude mice and BD-1X rats injected via the intrasplenic route may also have developed lung metastases, but the rapid formation of fatal ascites precluded longer observation.

A troubling feature of human colon carcinoma is the formation of recurrent tumor growth at sites of resection, particularly along suture lines (Edynak, 1974). The microenvironment of wounded tissue appears to promote tumor growth (Pozharisski, 1975). A similar phenomenon was observed with K12/TR cells. Accelerated tumor growth was evident at the wound site of nude mice 14 days after i.p. injection of K12/TR cells. A number of possible mechanisms could account for this effect, including the local release of growth factors and/or the formation of a substratum for cell adhesion at the injury site. Much additional work will be required to unravel the molecular basis of this observation. However, this protocol may offer a useful model to study the mechanisms involved in promotion of tumor growth by non-specific tissue injury.

In summary, the epithelial origin of the K12/TR cell line, and the reproducible patterns of spontaneous metastasis produced via a variety of routes of inoculation, make it an attractive system for studying particular aspects of metastatic colon carcinoma. In the absence of other well-characterized syngeneic models, the ability to study this tumor in immunocompetent, syngeneic hosts and *in vitro* provides a means of analyzing the efficacy of biological response modifiers and autochthonous tumor cell vaccines as novel therapeutic strategies.

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