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Original Research

Failure-to-Thrive Syndrome Associated with Tumor Formation by Madin–Darby Canine Kidney Cells in Newborn Nude Mice

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Tumors that formed in newborn nude mice that were inoculated with 10^7 Madin–Darby canine kidney (MDCK) cells were associated with a failure-to-thrive (FTT) syndrome consisting of growth retardation, lethargy, weakness, and dehydration. Scoliosis developed in 41% of affected pups. Pups were symptomatic by week 2; severely affected pups became moribund and required euthanasia within 3 to 4 wk. Mice with FTT were classified into categories of mild, moderate, and severe disease by comparing their weight with that of age-matched normal nude mice. The MDCK-induced tumors were adenocarcinomas that invaded adjacent muscle, connective tissue, and bone; 6 of the 26 pups examined had lung metastases. The induction of FTT did not correlate with cell-line aggressiveness as estimated by histopathology or the efficiency of tumor formation (tumor-forming dose 50% endpoint range = $10^{2.8}$ to $10^{7.5}$); however, tumor invasion of the paravertebral muscles likely contributed to the scoliosis noted. In contrast to the effect of MDCK cells, tumor formation observed in newborn mice inoculated with highly tumorigenic, human-tumor–derived cell lines was not associated with FTT development. We suggest that tumor formation and FTT are characteristics of these MDCK cell inocula and that FTT represents a new syndrome that may be similar to the cachexia that develops in humans with cancer or other diseases.

Abbreviations: FTT, failure-to-thrive; MDCK, Madin–Darby canine kidney; TPD_{50}, tumor-producing dose $\log_{10}$ 50% endpoint.

The Madin–Darby canine kidney (MDCK) cell line was established in 1958 from the kidney of a cocker spaniel.[6,16] Since 1962, this cell line has been an important reagent for the isolation and study of influenza viruses[8,22,31] and, more recently, for the development and manufacture of influenza virus vaccines.[3,7,19] MDCK cells are polarized, epithelial cells that exhibit properties of renal tubular epithelium and have been used as in vitro models to evaluate renal tubular functions.[24,36] Due to their apparent lack of expression of a tumorigenic phenotype in rodents,[25] MDCK cells have also been used to study neoplastic processes including epithelial-to-mesenchymal transition[23,27,28] and to assess the effects of viral oncogenes and chemical carcinogens on their phenotype.[13,32]

The results of studies that evaluate the ability of MDCK cells to form tumors in vivo have varied. Early studies found that these cells could produce tumors in chicken embryos but not in mature BALB/c nude mice.[14] In contrast, MDCK cells formed progressively growing adenocarcinomas in newborn BALB/c nude mice, but tumor growth ceased as the pups approached maturity.[25] More recently, 2 different sublines of MDCK cells developed by independent groups were shown to be tumorigenic in athymic nude mice; but the incidence of tumor formation did not correlate with cell dose.[33-35]

As an initial approach to the study of neoplastic development in cells in culture, we evaluated the ability of MDCK cells to form tumors in athymic nude mice. We previously described the tumor-forming capacity of MDCK cells from different lots obtained from ATCC.[21] That study revealed that MDCK cells from each of these lots formed tumors efficiently in adult and newborn nude mice, but the capacity of the cells to form tumors differed from lot to lot. During the initial experiments on MDCK cell tumor-forming efficiency in newborn nude mice, we observed what appeared to be a syndrome whose symptoms included tumor formation and disrupted growth leading to a failure-to-thrive (FTT) condition manifested by morbidity that required euthanasia of those pups most severely affected. During the study on the development of FTT, we found that the FTT syndrome occurred in newborn nude mice inoculated with 3 different sublines of MDCK cells. The current report describes an FTT syndrome associated with the formation of tumors by 10^7 MDCK cells in newborn, athymic, nude mice.

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Materials and Methods

Cells and cell culture. The MDCK vial 1 and vial 2 cells were obtained from ATCC (Chantilly, VA; NBL2 catalog no. CCL-34, lot no. 3563161 frozen January 2004 and lot no. 4398972 frozen January 2006) at tissue culture passage 55 and 56, respectively. Working cell banks of these 2 lots were established at passages 57 and p58, respectively. Cells used for inoculation during this study were prepared from cells propagated from these cell banks and cultured to passages 62 to 65 to generate the numbers of cells required for inoculation. The third subline of MDCK cells, designated MDCK-DVP, is a line of MDCK cells that was available within the Division of Viral Products (Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD). A working cell bank of MDCK-DVP cells was prepared at passage 25. MDCK-DVP cells for inoculation were prepared from this cell bank at passages ranging from 26 to 28. The relationship between the passage level of the MDCK-DVP cells and that of the MDCK cells obtained from the ATCC is unknown.

Additional human cell lines used in this study were obtained from the ATCC: A549 cells (catalog no. CCL185, lot no. 58016241), HeLa cells (catalog no. CCL2, lot no. 4965434), HT1080 cells (catalog no. CCL121, lot no. 6805248), MDA-MB-231 cells (catalog no. HTB-26, lot no. 58385316), and HepG2 cells (HB8065). MDCK vial 2 cells were grown by using Eagle's Minimum Essential Medium with 2 mM L-glutamine (ATCC) supplemented with 10% FBS (Hyclone, Logan, UT.) All of the other cell lines were propagated using DMEM (Mediatech, Manassas, VA) supplemented with 10% FBS and 2 mM L-glutamine (Mediatech). These cells were devoid of 26 rodent agents (ectromelia virus, epizootic diarrhea of infant mice virus, Hantaan virus, K virus, H1 virus, Kilham rat virus, lymphocytic choriomeningitis virus, lactate dehydrogenase elevating virus, murine adenovirus, mouse coronavirus, mouse hepatitis virus, mouse norovirus, mouse parvovirus, mouse thymic virus, minute virus of mice, polyoma virus, pneumonia virus of mice, rat cytomegalovirus, rat coronavirus–sialodacryoadenitis virus, reovirus 3, rat minute virus, rat parvovirus, Sendai virus, Seoul virus, Theiler murine encephalomyelitis virus-like virus, Theiler murine encephalomyelitis virus strain GDVII), and *Mycoplasma* species (Impact VIII PCR Profile, Research Animal Diagnostic Laboratory, Columbia, MO).

Animals. Heterozygous (NCr$^{nu/+}$) female mice bred with homozygous (NCr$^{nu/nu}$) male mice were obtained from the National Cancer Institute (Frederick Cancer Research Facility, Frederick, MD) as pregnant females at 16 to 17 d of gestation. The pregnant mice were housed singly in microisolation and individually ventilated caging with sterilized bedding (Quality Lab Products, Elkridge, MD). Mice were maintained on sterilized water and mouse chow (ProLab IsoPro 3000 Irradiated Diet containing 22% protein; LabDiet, St Louis, MO). Mice were exposed to a 12:12-h light:dark cycle in the SPF (including *Helicobacter* and norovirus) facilities of the Division of Veterinary Services (Center for Biologics Evaluation and Research). The ratio of male:female pups in the 145 newborn mice used in this study was 64:62; 19 of these 145 pups were asymptomatic mice and their sex was not determined. The health status of these animals was monitored daily. The animal facility is SPF and AAALAC-accredited. The protocol under which these studies were performed was approved by the Center for Biologics Evaluation and Research IACUC.

Tumorigenicity assays. The details of the tumorigenicity assays in nude mice have been described.[21] Briefly, suspensions containing 10^7 cells in PBS (pH 7.0) were inoculated subcutaneously in the dorsal region of the thorax over the scapulae in 0.1 mL by using 1-mL syringes with 27-gauge, 0.5-in. needles. With the exception of the time-course assay involving pups as old as 120 h, newborns were inoculated within 48 h of birth. Inoculated pups were evaluated initially by clinical observation, and progressive tumor growth was determined according to 2-dimensional measurements obtained by using a VWR Digital Caliper (0 to 150 mm; VWR International, Radnor, PA). Due to the unexpected appearance of the FTT syndrome in newborn nude mice inoculated with MDCK cells, it was necessary to establish the basic characteristics of this syndrome and ensure that this disease did not pose a threat to the vivarium. After resolving these issues, specific parameters (described following) were established to characterize the FTT syndrome on the basis of weight development by the inoculated pups. These parameters were applied to the final experiment involving the MDCK vial 1 cells and to all of the newborns inoculated with the MDCK vial 2 and MDCK-DVP cells. Inoculated newborns were marked by tattooing as soon as the homozygous nude (*nu/nu*) pups could be distinguished from their haired (heterozygous *nu*/+) littermates (about 4 d of age). Heterozygous (*nu*/+) pups were removed from the litter when the mice were weaned at 21 to 24 d. The nude newborns were followed by clinical observation, and body weight measurements were recorded every 2 to 3 d, from age 4 to 40 d. Mean weights were determined by using Excel (Microsoft, Redmond, WA). Pups demonstrating disruption of normal growth were submitted live to the Division of Veterinary Resources Diagnostic Laboratory (NIH, Bethesda, MD) for necropsy.

Development of a standard curve of normal weight by using uninoculated pups. To compare the body weights of age-matched controls with those of pups that were inoculated with MDCK cells, we determined normal weight curves for the athymic nude mice over the first 84 d of life. The weights of uninoculated, nude pups from a cohort of 5 litters containing 26 nude pups were obtained from age 4 to 84 d. Body-weight measurements were recorded 3 times each week, as described in the previous section. To confirm the initial data, weight curves were determined within 24 mo by using a second cohort of 16 nude pups, weighed every 2 to 3 d for 30 d. Because these new data were similar to the original data, they were combined with the data on the 26 pups, for a final tabulation of average weights on 42 newborn nude mice throughout maturation. To confirm that inoculation of newborn mice within 48 h of birth with a 0.1-mL inoculum did not affect development, weights of newborn mice injected with PBS were compared with the weights of normal mice and those of mice injected with MDCK cells. Tattooed pups injected with PBS were weighed every 2 to 3 d for 40 d. The data on the PBS-injected mice included the weights of 36 pups from 7 litters in 3 replicate experiments. Weight curves and statistical comparisons (2-tailed *t* test; $P < 0.05$) were generated by using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Pre- and postmortem evaluations of newborns inoculated with MDCK cells. Mice were weighed and observed every other day for 30 to 40 d for evidence of symptoms suggestive of illness or abnormal growth and development, including the onset of tumor formation, failure to grow and gain weight, lethargy, weakness (trembling while moving about), dehydration, and scoliosis. Symptomatic mice were euthanized by CO_2 inhalation, necropsied, and evaluated for signs of disease, tumor formation, and metastasis. Portions of tumors and organs (liver, kidney, spleen,

heart–lungs, and brain) were fixed in 10% formalin for histopathologic evaluation. Data obtained at necropsy and by clinical observation were recorded in Excel spreadsheets (Microsoft, WA). Attempts at blood collection for CBC analysis and serum chemistries from emaciated mice were generally unsuccessful.

Results

Tumor formation by MDCK cells and development of the FTT syndrome in newborn nude mice. Previous studies in our laboratory found that newborn nude mice can be more sensitive than adult nude mice to tumor formation by virus-transformed and spontaneously transformed rodent cells that were propagated in tissue culture.[15,17] One group of authors has reported that MDCK cells formed adenocarcinomas in BALB/c newborn nude mice that regressed as the mice matured.[25] In light of these findings, we evaluated the ability of MDCK cells to form tumors in newborn and adult athymic nude mice.[21] During these studies, some of the newborn nude mice inoculated with doses of 10^7 cells/mouse exhibited severe retardation of growth (Figures 1 A and B). Necropsy (gross examination and tissue histopathology) of pups inoculated with 10^7 MDCK cells indicated that the FTT syndrome was associated with the MDCK cell inoculations. In addition, a time-course of inoculation showed an age-related correlation with the development of FTT. Pups inoculated when 24 or 48 h old were symptomatic, with FTT in all 5 or 6, respectively, of the mice inoculated, compared with FTT in none of the 6, 8, or 5 mice inoculated at ages of 72 h, 96 h, or 120 h, respectively.

To further characterize the FTT syndrome, 5 human cell lines derived from human tumors (HeLa, A549, HT1080, MDA-MB-231, HepG2) known for their aggressive behavior were evaluated for their ability to induce FTT in newborn nude mice inoculated at 24 to 48 h of age with 10^7 cells. The tumor-producing dose 50% endpoint (TPD_{50}) in dose–response tumorigenicity assays of these cell lines ranged from $10^{4.9}$ to $10^{3.5}$ in adults and from $10^{2.2}$ to $10^{3.7}$ in newborns (TPD_{50} adults/newborns: HeLa cells, $10^{4.9}/10^{3.7}$; A549 cells, $10^{3.5}$/not tested; HT1080 cells, $10^{4.4}/10^{2.2}$; TPD_{50} values for MDA-MB-231 and HepG2 were not determined]. All 65 newborn mice inoculated with these human cell lines developed rapidly growing tumors, but none of the tumor-bearing mice developed clinically observable signs of FTT (Table 1).

Criteria for the diagnosis of the FTT syndrome. Initially, the marked growth retardation exhibited by critically ill pups provided the basis for the diagnosis of FTT syndrome in these animals (Figure 1). Clinical observation of growth-retardation, as well as lethargy, weakness, dehydration, and, in some cases, scoliosis of the thoracic spine (Figure 1 B) readily distinguished sick pups from their asymptomatic littermates. These observations and the weights of the sick mice at euthanasia were the criteria used during the initial portion of the study that involved MDCK vial 1 cells. However, as the study progressed, we recognized that failure to grow and gain weight, rather than signs of systemic illness, were the most reliable symptoms of the FTT syndrome. To document growth retardation, starting at approximately day 4, newborn nude mice were marked by tattooing and weighed at 2- to 3-d intervals during the first 30 to 40 d after birth. This method for following maturation was applied to the final sets of experiments performed by using MDCK vial 1 cells and to all of the experiments involving MDCK vial 2 and MDCK-DVP cells. The weights, at euthanasia, of newborn nude mice inoculated with MDCK cells compared with the average weights for age-related,

Figure 1. (A). Female pup (age, 17 d) with moderate FTT (weight, 4.4 g) inoculated with 10^7 MDCK vial 1 cells at age 24 to 48 h. Average weight for pups of this age is 10.4 g. The food pellet in the picture measures 30 mm × 16 mm. (B) Pups (age, 19 d) inoculated with 10^7 MDCK vial 1 cells at age 24 to 48 h. The smaller pup has what appears to be moderate FTT with scoliosis; the larger pup appears to be disease-free. Neither of these pups has a visible tumor.

uninoculated and PBS-inoculated control nude mice (Figures 2 A and B) enabled us to distinguish symptomatic from asymptomatic mice and to create disease categories. Mice weighing more than 80% of the average weight of age-matched control mice were classified as having no disease; mice with mild FTT weighed between 60.1% to 80.0% of the control weight; mice with moderate FTT weighed between 40.1% to 60% of the control weight; and mice with severe FTT weighed 40% or less of the average weight for that age. The curve generated for each disease category differed significantly ($P < 0.05$) from the weight curve of control mice (Figure 2 A). The weights of these maturing pups obtained either at euthanasia or at 2- to 3-d intervals illustrated the development of severe, moderate, and mild FTT.

Characterization of the systemic disease in newborn nude mice inoculated with MDCK cells. In the first 4 experiments with MDCK vial 1 cells, 67% of the inoculated pups were heterozygous (*nu*/+) haired mice. Over the 180-d observation period, the haired mice failed to develop tumors, and their general health and development appeared normal. Thereafter, the haired pups were routinely euthanized when the mice were weaned, usually at 21 to 24 d of age.

Table 1. Incidence of FTT syndrome in newborn nude mice among 19 independent assays of canine MDCK vial 1, vial 2, and MDCK-DVP cells and cells from human cell lines known to express highly tumorigenic phenotypes

Cell line (no. of experiments)	TPD_{50} (log_{10}) in newborn mice	No. of nude pups (no. of litters)	No. of pups with tumors/ total no. of pups	Incidence of FTT				
				None	Mild	Moderate	Severe	Overall (%)
MDCK vial 1 (8)	3.7	79 (28)	76/79	37/79	10/79	22/79[a]	10/79[a]	42/79 (53)
MDCK vial 2 (3)	2.5	35 (8)	34/35	9/35	8/35[b]	15/35	3/35	26/35 (74)
MDCK-DVP (3)	7.3	31 (16)	10/31	21/31	3/31	4/31	3/31	10/31 (32)
HeLa (1)	3.5	9 (2)	9/9	9/9	0/9	0/9	0/9	0/9
A549 (1)	not determined	10 (3)	10/10	10/10	0/10	0/10	0/10	0/10
HT1080 (1)	2.2	23 (5)	23/23	23/23	0/23	0/23	0/23	0/23
MDA-MB-231 (1)	not determined	16 (4)	16/16	16/16	0/16	0/16	0/16	0/16
HepG2 (1)	not determined	7 (3)	7/7	7/7	0/7	0/7	0/7	0/7

[a]No tumors were apparent according to clinical observation or necropsy in 1 pup with moderate FTT and 2 pups with severe FTT and inoculated with MDCK vial 1 cells and in 1 pup with mild FTT and inoculated with MDCK vial 2 cells.

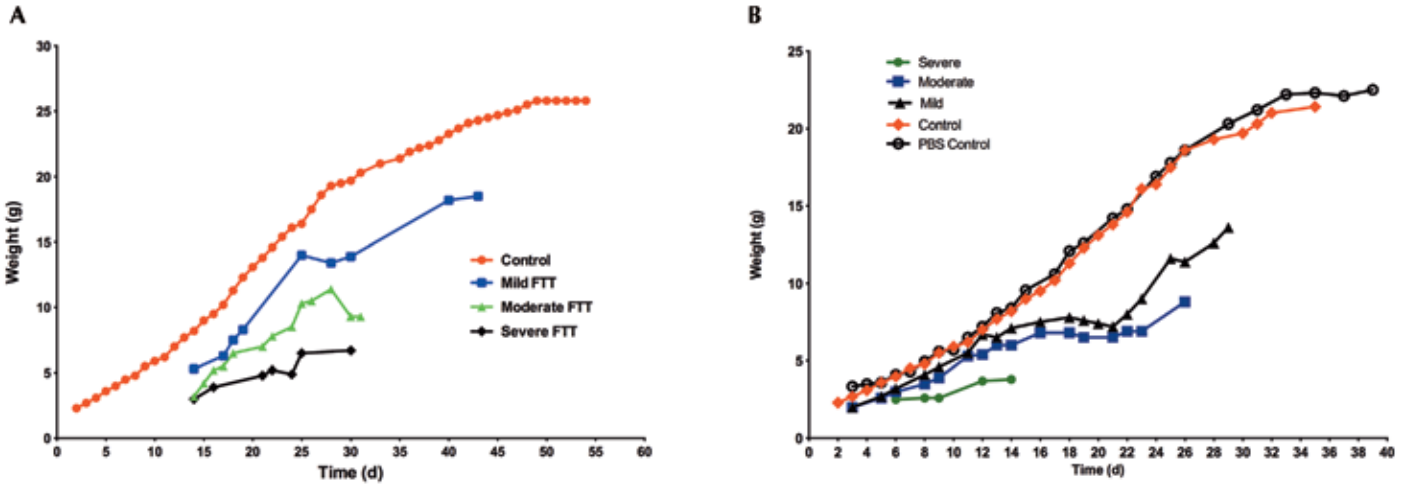

Figure 2. (A) Weight curves during the first 45 d of life that compare the weights (recorded at necropsy) of those nude mice (inoculated with MDCK cells vial 1, vial 2, and MDCK-DVP; 10^7 cells/mouse) that developed mild, moderate, or severe FTT with the weights of control pups in these experiments. The weight curves generated according to each disease category differed significantly (2-tailed assays) from the weight curve of the control mice: mild FTT compared with control, $P = 0.0011$; moderate FTT compared with control, $P = 0.0001$; severe FTT compared with control, $P = 0.0001$. (B) Weight development during the first 35 to 40 d of life of pups inoculated with MDCK vial 2 cells and PBS. These mice were marked by tattooing at approximately 4 d of age. Each mouse was weighed every 2 to 3 d; weights were averaged according to each category of the FTT syndrome. The curves generated according to disease category differed significantly (2-tailed assays) from the weight curve of control mice: mild compared with control, $P = 0.0192$; moderate compared with control, $P = 0.0012$; severe compared with control, $P = 0.0084$. The curve of the mice injected with PBS did not differ ($P = 0.58$) from that of the uninjected normal controls.

A total of 19 experiments in this study involved 210 newborn nude (*nu/nu*) mice. The mice were inoculated subcutaneously in the dorsal region at the base of the neck above the scapulae with 10^7 cells/mouse within 48 h after birth. MDCK cells (vial 1, vial 2, MDCK-DVP) were inoculated into 145 newborn nude mice; cells from the 5 human tumor cell lines were inoculated into 65 newborn nude mice. Of the 145 newborn nude mice inoculated with MDCK cells, 67 had no FTT; the remaining 78 were diagnosed with FTT (Tables 1 and 2). Gross pathologic examinations (necropsies) were performed on 110 of the 145 newborns that, by clinical observations, had FTT. Necropsies were performed on 63 of the 79 (80%) mice injected with MDCK vial 1 cells, all 37 (100%) of the mice injected with MDCK vial 2 cells, and 10 of the 31 (32%) mice injected with MDCK-DVP cells.

Of the 145 MDCK-cell–inoculated mice, 78 (54%) developed FTT according to weight measurements and clinical observations, whereas the remaining 67 (46%) were asymptomatic. Of the 78 symptomatic mice, 74 (95%) had tumors at the site of inoculation that ranged from 2 to 10 mm in diameter, whereas 4 failed to exhibit detectable tumor formation (by visual observation, palpation, or necropsy). In contrast, tumors that developed in the 67 (46%) pups that failed to develop FTT grew progressively to 20 mm within 2 mo, at which time the mice were euthanized.

On the basis of diagnosis by weight, 21 of the 145 (14%) pups inoculated developed mild FTT over the first 14 to 43 d of their life; their weights were between 5.3 g on day 14 and 18.5 g on day 43 (average weight for control mice: age 14 d, 7.7 g; age 43 d, 24.3 g). Tumors ranging from 2 to 10 mm developed in 20 of 21 (95%) of these mice; the remaining mouse (5%) did not have a detectable tumor (Table 2). Moderate FTT developed in 41 of the 145 (28%) newborn mice, with 40 of 41 (98%) having tumors that ranged from 2 to 10 mm in diameter at ages 14 to 31 d, with weights of 3.2 g on day 14 and 11.6 g on day 31 (average weight of control on day 31, 20.3 g). Only 1 of the 41 (2%) mice with moderate FTT

Table 2. Parameters of the FTT syndrome induced in newborn nude mice by 3 sublines of MDCK cells at 10^7 cells/mouse

	No. of mice	Age range (d)	Weight range (average normal weight; g)	No. of pups with tumors/ total no. of pups (%)	Tumor diameter (mm)	No. of pups with scoliosis/ total no. of pups (%)
No disease	67[a]	4–180	3.0–26.5 or more	46/67 (69)	5–20	0/67 (0.0)
Mild FTT	21	14–43	5.3–18.5 (7.7–24.3)	20/21 (95)[b]	2–10	7/21 (33)
Moderate FTT	41	14–31	3.2–11.6 (7.7–20.3)	40/41 (98)[b]	<5–10	23/41 (56)
Severe FTT	16	14–30	3.0–6.7 (7.7–19.7)	14/16 (88)[b]	<5–6	4/16 (25)
Total	145	4–180	3.0–26.5 or more	123/145 (85)	<5–20	34/145 (23)

[a]No FTT observed: newborns matured until they were euthanized with tumors approximately 20 mm or when the experiment was terminated at 180 d.
[b] No tumors were apparent according to clinical observation or necropsy in 1 pup with moderate FTT and 2 pups with severe FTT and inoculated with MDCK vial 1 cells and in 1 pup with mild FTT and inoculated with MDCK vial 2 cells.

lacked tumor development (Table 2). During similar 14 to 30 d periods of maturation, 16 of the 145 (11%) inoculated pups developed severe FTT, with weights of 3.0 g on day 14 and 6.7 g on day 30 (average control weight on day 30 was 19.7 g). Of the 16 pups with severe FTT, 14 (88%) had tumors as large as 6 mm; tumors were not detected in the remaining 2 (13%) mice (Table 2).

FTT was associated with the inoculation of MDCK vial 1, vial 2, and DVP cells. In the 8 experiments in which 79 neonatal mice were injected with 10^7 MDCK vial 1 cells (TPD_{50} $\log_{10}$ adults/newborns, 5.2/3.7), 75 (95%) mice developed tumors, and FTT developed in 42 of the 75 (56%) tumor-bearing mice (Table 1). Of the 75 tumor-bearing mice, 37 (49%) mice had no FTT, 10 (13%) mice had mild FTT, 22 (29%) had moderate FTT, and 10 (13%) had severe FTT. According to clinical observations and weight data, 1 pup with moderate FTT and 2 pups with severe FTT after inoculation with MDCK vial 1 cells failed to develop detectable tumors (Table 2).

Of the 35 newborn mice inoculated with the MDCK vial 2 cells (TPD_{50} $\log_{10}$ adults/newborns, 4.4/2.1), 34 (97%) developed tumors and 26 (74%) developed FTT. Of the 34 mice that developed tumors, 8 (24%) developed mild FTT, moderate FTT was observed in 15 (44%), and severe FTT occurred in 3 (9%).

MDCK-DVP cells (weakly tumorigenic cells; TPD_{50} $\log_{10}$ adults/newborns, >7.5/7.3) formed tumors in only 10 of the 31 mice inoculated. FTT occurred in all 10 mice with tumors with mild FTT in 3 (30%), moderate FTT in 4 (40%), and severe FTT in 3 (30%).

Development of scoliosis in newborns with FTT syndrome. Of the 78 mice in the study that developed FTT, 34 developed scoliosis as a component of the syndrome (Figure 1 B, Table 2). Scoliosis, as determined by clinical observation and at necropsy, occurred in 19 of 79 (24%) pups inoculated with vial 1 MDCK cells, in 14 of the 35 (40%) pups inoculated with vial 2 MDCK cells, and in 1 of the 31 (3%) pups inoculated with MDCK-DVP. In the 34 mice inoculated with MDCK vial 1, vial 2, or MDCK-DVP cells that developed scoliosis, 7 (21%) were diagnosed as having mild FTT, 23 (68%) had moderate FTT, and 4 (12%) had severe FTT. None of the pups that failed to develop FTT developed scoliosis; however, 2 of the 79 (3%) pups inoculated with 10^7 MDCK vial 1 cells developed FTT and scoliosis in the absence of detectable tumors.

Histopathologic findings in mice with the FTT syndrome. The tumors that developed in newborn mice inoculated with MDCK vial 1 and vial 2 cells were adenocarcinomas that exhibited similar characteristics. These tumors were composed of tubular-like structures, separated by bands of spindle cells (Figure 3 A) and solid areas of epithelial cells, with areas of necrosis and mineralization. Tumors formed by MDCK vial 1 and vial 2 cells were aggressive, with mitotic figures in both epithelial and spindle cells (Figure 3 B), and invaded the adjacent muscles, including the paravertebral muscles, bone including the spinal cord (Figures 3 A and C), and dermis. Necropsy data documented that 3 of the 10 (30%) mice injected with MDCK vial 1 cells and 3 of the 16 (19%) mice inoculated with MDCK vial 2 cells had metastases in their lungs (Figure 3 D). Small areas of inflammation were present within the parenchyma of some tumors. Adenocarcinomas formed by MDCK-DVP cells resembled the adenocarcinomas formed by MDCK vial 1 and vial 2 cells but generally were less invasive. The majority (7 of 8, 88%) of the MDCK-DVP cell tumors also had necrosis; however, tubule formation was less frequent than that observed in the tumors formed by MDCK vial 1 or vial 2 cells. None of the 10 mice bearing MDCK-DVP tumors had detectable metastases in their lungs. Tissues from the other organs including the heart–lung, liver, spleen, kidney, gastrointestinal tract, and brain including the pituitary were normal. No evidence of inflammation was detected. Tissues were not available for serial sectioning from the 4 mice (2 of which developed scoliosis) that developed FTT without developing detectable tumors.

Discussion

MDCK cells have several characteristics that contribute to their broad use, including their ability to support replication of a broad range of influenza virus isolates to high titer, their resemblance to polarized epithelial cells and renal tubular epithelial cells, their epithelial-to-mesenchymal transition characteristics, and their neoplastic phenotype, which can be evaluated by modifications with viral and cellular oncogenes. Our laboratory evaluated the tumor-forming capacities of different lots of MDCK cells and used these findings to expand the diversity of MDCK cell characteristics to include differences in tumor-forming efficiency.[21] The current report describes an FTT syndrome induced by MDCK cells in newborn nude mice. Clinically, this FTT syndrome resembles the cachexia that can develop in humans with certain types of malignancies.

To define this FTT syndrome, we developed a diagnostic procedure based on the weights of newborn nude mice inoculated with MDCK cells compared with the average weights of uninoculated age-matched, newborn nude mouse controls over the course of their maturation. By comparing the weight of MDCK-cell–inoculated mice with the average weights of uninoculated mice of the same age, 4 categories of disease could be distinguished. These categories ranged from no FTT (>80% of average control weight) in 67 of 145 (46%) mice; mild FTT (60.1% to 80% of average

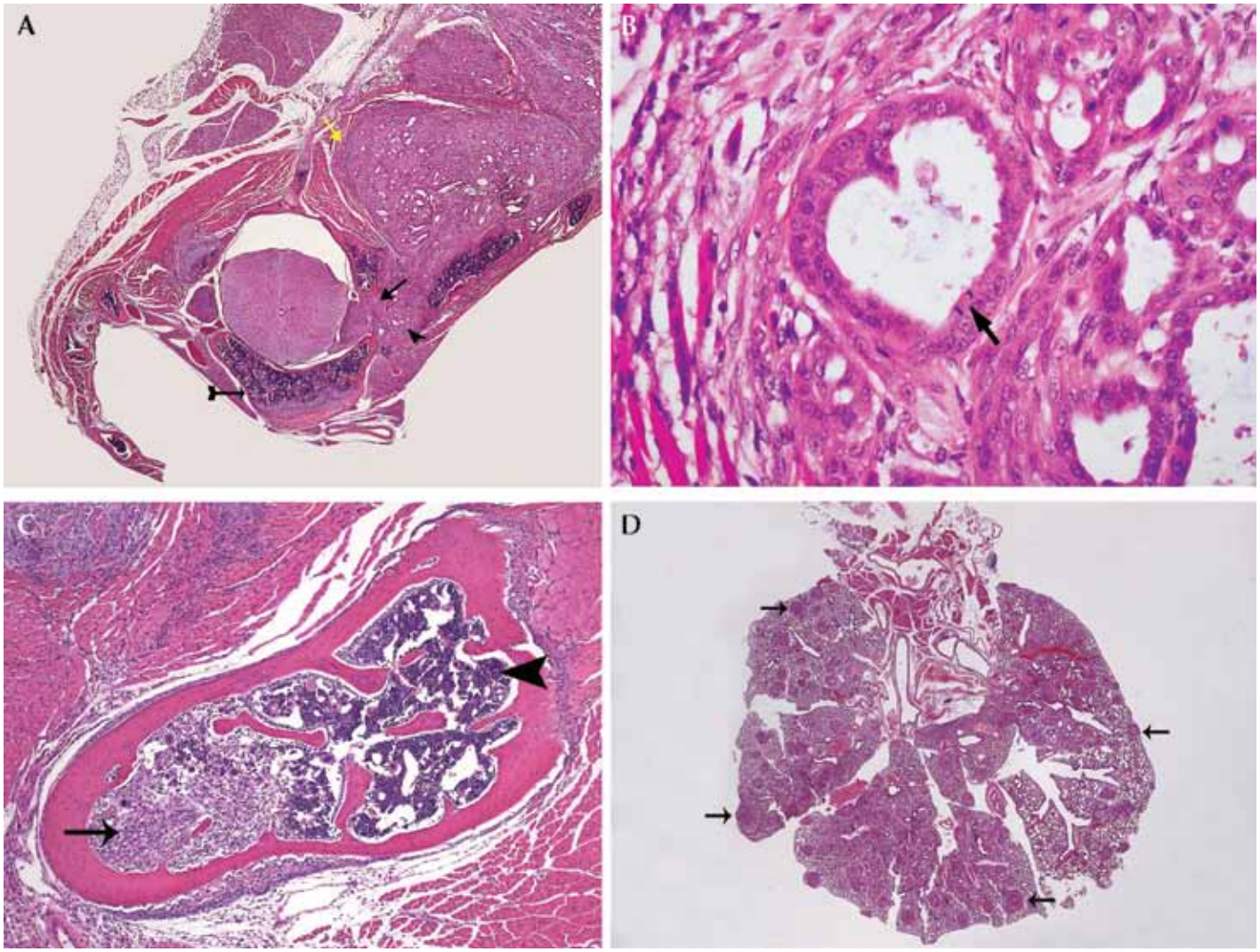

Figure 3. Sections of primary tumors and lung metastases in nude mice inoculated as newborns with MDCK vial 1 or vial 2 cells. (A) MDCK vial 2 cell adenocarcinoma with tubules and spindle cell areas. There is invasion into paravertebral muscles (yellow arrow) and vertebral column (black arrow) and between a vertebral body (tailed arrow) and adjacent rib (arrowhead). This mouse had moderate FTT and scoliosis. Magnification, 1.25×. (B) MDCK vial 2 cell adenocarcinoma with mitotic figure (arrow) in tubular epithelial cells . Magnification, 40×. (C) MDCK vial 1 cell adenocarcinoma invading bone. Neoplastic cells are within marrow on the left (arrow), with normal marrow on the right (arrowhead). Magnification, 5×. (D) MDCK vial 2 cell pulmonary metastases (arrows). Magnification, 2.5×. Hematoxylin and eosin stain.

control weight) in 21 of 145 (14%) mice; moderate FTT (40.1% to 60% of average control weight) in 41 of 145 (28%) mice; to severe FTT (40% or less of the average weight of control mice) in 16 of 145 (11%) mice. A component of the clinical features of this FTT syndrome was the development of scoliosis of the thoracic spine in 34 of 82 (41%) newborns across the spectrum of FTT.

Concerning the relationship between the tumor-forming efficiency of our 3 MDCK cell sublines and the development of FTT in mice bearing these tumors, the syndrome developed most frequently (26 of 34 mice, 76%) in newborns inoculated with MDCK vial 2 cells, the cells that formed tumors most efficiently (TPD_{50} log_{10} adults/newborns, 4.4/2.1), suggesting that tumor-cell aggressiveness, as defined by the efficiency of tumor formation and measured by the TPD_{50}, was a key factor in initiating the FTT syndrome. MDCK-DVP cells, which failed to form tumors in adult nude mice (TPD_{50} log_{10}, greater than 7.5) and inefficiently formed tumors in newborns (TPD_{50} log_{10}, 7.3) produced FTT in 10 of the 31 (32%) pups studied. Interestingly, only 1 of the 10 (10%) newborns that developed FTT after inoculation with MDCK-DVP cells developed scoliosis compared with 19 of the 79 (24%) and 14 of the 35 (40%) inoculated with MDCK vial 1 cells and vial 2 cells, respectively. Given the failure of the 5 human tumor-derived cell lines—all known to be aggressive in nude mice—to induce FTT and the data from both highly aggressive (vial 2 cells) and weakly aggressive (DVP cells) MDCK cells, cell line aggressiveness does not seem to correlate with the development of FTT in newborn nude mice. These observations suggest that mechanisms other than tumor-cell aggressiveness, as measured by TPD_{50} values, contribute to the development of the FTT syndrome. Supporting this suggestion is the observation that 4 of the 68 (6%) pups inoculated with MDCK vial 1 or vial 2 cells developed mild, moderate, or severe FTT but did not develop tumors that were visible or

detectable at necropsy. These observations suggest that tumor formation, tumor-cell aggressiveness as measured by TPD_{50} values, invasion, and metastasis either alone or collectively are not solely responsible for the development of the FTT syndrome.

Given the finding that 94% of mice with FTT had tumors, this syndrome in newborn mice appears to be associated with MDCK-cell inoculation and tumor formation. However, 4 of the 145 pups developed FTT without developing tumors detectable by observation or at necropsy. Tissues required to evaluate the formation of inconspicuous, invasive tumors by examining serial sections through the paraspinal regions of these mice were unavailable for histopathology. Reinforcing the implication that the FTT syndrome in newborn nude mice is an MDCK-cell–associated disease are data associating this entity with 3 different sublines of MDCK cells. In contrast, 5 cell lines established from human tumors—all recognized for their ability to form tumor xenografts efficiently, to invade locally, and to metastasize[9,26,29,40-42]—failed to induce FTT when they formed tumors in newborn nude mice inoculated with 10^7 cells. So far as we are aware, FTT as a syndrome associated with the development of MDCK cell tumor xenografts in newborn nude mice has not yet been described.

From the histopathologic findings associated with this syndrome in mice, tumors formed by the MDCK sublines were adenocarcinomas consisting of epithelial lined tubular and cystic structures separated by spindle cells and/or solid sheets of epithelial cells. Cells from tumors formed by MDCK vial 1 and MDCK vial 2 cells invaded into adjacent adipose, muscle, and bone. Tumor invasion of the paravertebral muscles likely contributed to the development of scoliosis. Newborns inoculated with cells from MDCK vial 1 (3/10) and MDCK vial 2 (3/16) had lung metastases. A comprehensive evaluation of the histopathology of the FTT syndrome in newborn mice will require detailed study that is beyond the scope of this report. These studies are underway and will be included in other reports.

Cachexia-like syndromes have been reported in rodents with tumor transplants.[1,2,4,18,20,38,39] These reports include adult C57BL/6 mice or their derivatives (MC4-RKO mice) and Sprague–Dawley or Wister rats inoculated with the spontaneous LLC tumor cell line, the ESH chondrosarcoma transplantable tumor line, the MAC16 colon adenocarcinoma line, Walker 256 carcinosarcoma line, and the Yoshida AH130 hepatoma line, as well as mouse and rat models involving methylcholanthrene-induced sarcomas. Other models that have been described include the C26 colorectal carcinoma mouse model.[12,37] In addition, human melanoma cell xenografts in nude mice, a model that has been used to evaluate the effects of ghrelin expression on this disease, have been reported to induce a cachexia-like syndrome.[10,11] All of these models involved the use of adult animals. Whether the development of tumor xenografts in newborn nude mice inoculated with representatives of this group of tumor lines or tumor cell lines are associated with the development of an FTT syndrome similar to the one we noted with MDCK cells merits additional study.

The similarities between the MDCK cell tumor-induced FTT syndrome and cachexia in humans with malignancies is a point to be considered. Cancer-associated cachexia in humans presents as anorexia, weight loss, and muscle wasting and is associated with metabolic changes that differ from those associated with reduced food intake and starvation.[2,30] Cachexia associated with malignancies in children can result in growth failure,[5] a finding that appears to be replicated in the MDCK cell-induced FTT we have observed in these newborn nude mice. Several authors have reviewed animal models of cachexia.[2,4] Cachexia in humans is associated with metabolic changes including hypoalbuminemia—a feature we noted in our preliminary studies of mice with FTT. Additional studies are underway to further define the FTT syndrome associated with MDCK cell tumor development in newborn nude mice and to determine whether it could serve as a model of cachexia in humans with neoplasia.

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