Methylnitrosourea-induced Carcinoma in Organ-cultured Fetal Human Pancreas

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ABSTRACT

Explants from 12- to 14-week-old human fetal pancreases were organ cultured in a chemically defined medium and cultured for up to 12 months in the presence or absence of methylnitrosourea (MNU). Differentiation of the exocrine pancreas occurred in vitro, and explants cultured in the absence of MNU for 4 weeks or longer revealed normal acinar structures with zymogen granules. Ducts and ductules also developed normally. The undifferentiated tubular structures of the 12- to 14-week fetal pancreas were tumorigenic in nude mice. Tumor cells revealed a human karyotype and expressed duct cell surface markers. Cells derived from 4- to 5-month MNU-treated explants were tumorigenic in nude mice. Tumor cells revealed a human karyotype and expressed duct cell surface markers. The development of centroacinar and ductal cell markers followed 2 weeks later. MNU-treated explants showed minimal degeneration and necrosis. MNU caused early loss of apical cytoplasm and zymogen granules in acinar cells, resulting in dilation of acinar lumens, concomitant proliferation of cells bearing duct cell markers, and ductal hyperplasia. Enhanced foci of proliferation and carcinoma developed within 3 and 5 months of treatment, respectively. Cells derived from 4- to 5-month MNU-treated explants were tumorigenic in nude mice. Tumor cells revealed a human karyotype and expressed duct cell surface markers.

INTRODUCTION

Pancreatic carcinoma represents a major clinical problem affecting 22,000 individuals in the United States each year, carrying a mortality of more than 99% within 5 years after the initial diagnosis. The lack of adequate knowledge of the causes and factors influencing its biological behavior together with the difficulty in early detection contribute to its poor prognosis (2). Experimental pancreatic carcinogenesis in rodents (1, 3, 4, 14, 15), in organ-cultured rat pancreas (6, 8, 9), and in the organ culture model of fetal human pancreas (10, 11) suggests that environmental chemicals, and in particular nitrosocompounds, are possible factors in the development of human pancreatic carcinoma. A model of adult human pancreas carcinogenesis was reported from this laboratory in which pancreatic explants were cultured in a chemically defined medium. All exocrine cell types demonstrated at least a limited ability to proliferate. Diamethylnitrosamine and MNU3 were both carcinogenic but with varying rapidity of induction. It was further reported that the acinar cells in this model underwent degeneration and necrosis within 6 to 12 weeks even in the absence of carcinogens. In order to study the effects of chemical carcinogens on both acinar and ductal cells, an organ culture model of fetal human pancreas was developed. The present report describes the in vitro differentiation of fetal human exocrine pancreas, the induction of carcinoma by MNU, and the modulation of cytotypic cell surface markers during carcinogenesis in this model.

MATERIALS AND METHODS

Organ Culture. Expiants (1 cu mm) were prepared from fetal human pancreases. Four to 20 explants were placed on a 4- x 100-mm strip of Millipore filter (Millipore Corp., Catalog No. HAWP. 304-FQ). Up to 3 strips were floated on 5 ml of prewarmed (37°) medium in a roller tube (Belloco, Vineland, NJ; Catalog No. 7733-10535). The medium was a chemically defined one (7) modified by the addition of ascorbic acid (10 mg/liter) and bovine pancreas crystalline insulin (8 mg/liter) (both from Sigma Chemical Co.). The roller tubes were incubated at 37° in an atmosphere of 10% CO2 in air saturated with water vapor and rotated at 0.5 rpm. The medium was changed twice a week. Explants were cultured for up to 20 weeks.

Fetal Pancreas. Fetal pancreases from 12- to 14-week postgastrulinduced abortuses were obtained within 1 to 4 hr after delivery. The pancreas was removed aseptically and placed in prewarmed (37°) medium, and explants were prepared by parallel incisions perpendicular to the main pancreatic duct.

MNU. A stock solution (5 mg/ml) of MNU (Ash Stevens, Inc., Detroit, MI) was prepared in acetone and stored at −20°. At the initiation of culture, MNU (5 µg) was added to the culture medium to give a final concentration of 2 µg/ml, and 3 hr later explants were transferred to culture medium without MNU. Explants were treated twice a week according to the same protocol.

Microscopic Preparation. On each culture day 1 through 14 and each culture week 3 through 20, 16 to 20 MNU-treated and untreated explants were examined by light microscopy. Explants were fixed in Bouin’s fluid and embedded in paraffin; 2- to 4-µm-thick sections were stained with hematoxylin and eosin. Two control explants from each pancreas were also processed for light microscopy.

Fluorescence Microscopy. For fluorescence microscopy, sections were deparaffinized in xylene, hydrated gradually, and placed in PBS, pH 7.2 to 7.4, for 10 min followed by 20 min incubation in goat serum diluted with PBS (1:10) and 3 washes with PBS, all at room temperature. Sections were then incubated for 30 min at room temperature in pooled ascitic fluid rich in anti-duct cell surface IgG (HP-DU-1), diluted with PBS (1:200). This was followed by 3 washes in PBS, incubated in fluorescein-conjugated goat anti mouse IgG for 30 min at room temperature, washed with PBS, and examined for fluorescence.

Double Staining. Sections were first stained as above using HP-DU-1 antibody and fluorescein-conjugated goat anti-mouse IgG, washed in PBS, and incubated in normal rabbit serum diluted with PBS (1:10) followed by 3 washes with PBS. These sections were then incubated in monoclonal antibody to human acinar cell surface (AC1) for 30 min at room temperature, rinsed in PBS, incubated for 30 min in rhodamine-conjugated rabbit anti-mouse IgG, washed with PBS, mounted in glycerol, and examined for green and red fluorescence.

Monoclonal Antibody. The specificity of AC1 and HP-DU-1 antibodies for human pancreatic duct cell surface was previously reported (5, 13). The hybrid producing specific IgG was developed by the fusion of splenocytes from BALB/c mice, immunized with human acinar or ductal

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3 The abbreviations used are: MNU, methylnitrosourea; PBS, phosphate-buffered saline.

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cells, and mouse myeloma cells (SP2). The IgG-secreting cells were screened, and AC1- or HP-DU-1-producing cells were cloned. Peritoneal exudates, rich in specific antiacinic or antidualt cell surface IgG from BALB/c mice given i.p. injections of specific hybrids, were used in this study.

Antibody to Keratin. Commercial anti-keratin antibody (Accurate, Westbury, NY) was diluted with PBS (1:2, 1:4, 1:10, 1:20) and used for detection of keratin in tumors from nude mice. Optional dilutions (1:10) were used in fluorescence microscopy of sections from 6 independent tumors induced in nude mice. Sections were incubated in normal rabbit serum diluted (1:10) with PBS, rinsed in PBS, incubated in anti-keratin serum for 1 hr at room temperature, rinsed, and incubated in fluorescein-conjugated serum.

Electron Microscopy. Explants of tumor tissue from nude mice were fixed in 2.5% glutaraldehyde in 0.10 M cacodylate buffer (pH 7.4) to which had been added sucrose (1.7% w/v) and calcium chloride (0.02% w/v) for 1 hr, rinsed with cacodylate buffer, and postosmicated in 3% osmium tetroxide in 0.067 M s-collidine buffer (pH 7.4) for 1 hr. Specimens were gradually dehydrated in ethanol and were embedded in EponAraldite. Ultrathin sections were stained with lead hydroxide and/or saturated methanolic solution of uranyl acetate.

In Vivo Inoculation. Explants from pancreases at the initiation of culture, control explants, and MNU-treated explants were pooled at the end of each culture month, and cell suspensions from each group were inoculated s.c. in the dorsal aspect of the thorax of 4- to 8-week-old nude mice. Nudes (2 to 3 per time point) were inoculated with 0.5 ml saline solution, the cell concentration was adjusted to 2 x 10^7 viable cells/ml. Nude mice (2 to 3 per time point) were inoculated with 0.5 ml of cell suspension. The nude mice were followed for up to 16 weeks, and the tumor or inoculation sites were processed for light, fluorescence, and electron microscopy. Portions of tumors developed in 3 nude mice 10 weeks after s.c. injections of cell suspensions from 24-week MNU-treated explants were trypsinized and reinoculated in nude mice as above.

Karyotype Analysis. Primary cultures (48 hr) from tumor nodules developed in nude mice were used for chromosomal preparation and rapid banding (16). Twenty to 40 cells per specimen were analyzed.

RESULTS
Morphogenesis and Differentiation

Fetal pancreas varied considerably in their developmental stages from 12 to 14 weeks of gestation. Variations in the degree of differentiation were also noted in 12-week-old fetuses. Pancreas sections from the earliest stage seen in 12-week-old fetuses showed a few branching, intercalating tubules lined by flat cuboidal epithelium with relatively large nuclei and scanty cytoplasm, surrounded by loosely arranged mesenchymal cells with small, elongated nuclei and poorly stained cytoplasm (Fig. 1a). The ramifying tubules showed terminal dilation forming immature acini lined by columnar epithelium with basally located nuclei and minimal amounts of apical cytoplasm. Although zymogen granules were not noted in these cells, they were easily distinguishable from the flattened cuboidal epithelium of the undifferentiated tubules. Occasional endocrine islands were noted adjacent to the ramifying tubules. The columnar epithelium of these tubules showed no fluorescent staining with either AC1 or HP-DU-1 antibody. During the first 2 weeks of culture, there was proliferation of acinar cells; by the end of the first culture week, numerous AC1-positive cells became apparent (Fig. 1b). The columnar epithelium of the branching interconnecting tubules, however, showed no reaction with either antibody. The pancreas sections of 14-day-cultured explants revealed a preponderance of epithelial structures interspersed by a small amount of loosely arranged mesenchyme. By the end of the third culture week, the acinar cells showed a fair amount of apical cytoplasm, containing zymogen granules, completely filling some of the acinar lumens (Fig. 1c). Centroacinar cells became distinguishable from acinar cells by their affinity for HP-DU-1 antibody at the end of the third culture week. Ductal and ductular epithelium with basally located nuclei developed and became distinguishable from acinar cells and the undifferentiated tubular epithelium by their affinity for HP-DU-1 antibody (Fig. 1, d and e). Endocrine islets were less commonly seen and were unremarkable. Within the next 2 weeks of culture, more acinar structures developed; by the end of the sixth culture week, the explants were packed with acini lined by acinar cells with large numbers of zymogen granules. Some acinar lumens showed slight dilation, but the apical cytoplasm retained a large number of zymogen granules.

Central necrosis in explants cultured beyond 7 weeks became progressively prominent. At the periphery of explants, however, many well-preserved acini with zymogen granules, unremarkable ducts, and ductules were seen. Central necrosis and acinar cell degranulation were prominent features in explants cultured beyond 12 weeks. A few acini with zymogen granules, however, were seen in explants cultured for 10 to 12 months (Fig. 1, f and g).

MNU-treated Explants. Cultured explants treated with MNU showed considerable variation in the degrees of proliferation, morphogenesis, and morphological alterations with each pancreas. During the first 4 weeks of culture, treated explants grew and differentiated similarly to untreated explants; acinar cell proliferation occurred and a large number of acini were seen formed by zymogen-containing epithelium; ductules and ducts appeared unremarkable.

The first distinctive changes appeared in the fourth week of culture. These consisted of degeneration and necrosis of both acinar and centroacinar cells detectable ultrastructurally as increased electron density (Fig. 2a) and disruption of cytoplasmic organelles. Gradually, the dilation of acinar lumens, the loss of apical cytoplasm in many acinar cells, and the concomitant appearance of mitotic figures in the dilated acini lined by cells devoid of zymogen granules were noticeable by light microscopy. Double staining of sections from explants in the fourth week of culture with both anti-acinar and anti-ductal antibodies revealed dilated acini lined by AC1-positive cells showing surface and cytoplasmic fluorescence interspersed with cells showing neither acinar nor ductal cell surface markers and HP-DU-1-positive cells with extensive cytoplasmic processes covering the luminal surfaces of the acinar cells (Fig. 2, b and c). The proliferation of these cells together with their extensive cytoplasmic processes forming an HP-DU-1-positive core for an acinus became noticeable in the fifth culture week (Fig. 2, d and e).

The formation of duct-like structures with large hyperchromic nuclei and sharp angular cytoplasm projecting into their lumens (Fig. 2f) was a common feature in explants cultured for more than 5 weeks. The cells lining the ductules expressed duct cell markers (Fig. 2g) and were devoid of zymogen granules; a few showed ultrastructural changes (increase in electron density and degeneration of organelles) (Fig. 2f).
By the end of the sixth culture week, explants were composed predominantly of these duct-like structures lined mostly by columnar epithelium with preserved polarity.

In the period between 6 and 10 weeks of culture, multicentric foci of dilated hyperplastic ductules appeared, lined by cells with hyperchromatic nuclei often forming intraluminal papillary projections (Fig. 3a). Ultrastructurally, the cells contained segmented large nuclei, inconspicuous nucleoli, and relatively small amounts of cytoplasm devoid of zymogen granules (Fig. 3b). They expressed intense fluorescent staining with HP-DU-1 (Fig. 3c). In more than 90% (96 of 102) of explants examined between 8 and 12 weeks, the papillary projections on the surface of the explants were extensive and, in many foci, were confluent with the hyperplastic glands.

In the next stage (12 to 20 weeks), clusters of mitotic figures appeared within the hyperplastic glands. Whereas during the first 12 weeks of culture individual mitotic figures were present throughout the explants, clusters of 6 or more mitotic figures were noted in sections of MNUN-treated explants of 12 or more weeks of culture (Fig. 3d).

Forty-three % (123 of 285) of explants of 20 to 24 weeks showed foci of increased mitotic activity, 39% revealed more advanced stages of neoplastic proliferation forming irregular glands lined by atypical cells with loss of polarity, large nuclei, prominent nucleoli, and scanty cytoplasm with HP-DU-1-positive markers (Fig. 3, e to g) to anaplastic cells compatible with adenocarcinoma (Fig. 3h). These cells revealed large irregular nuclei, scanty cytoplasm, rare rough endoplasmic reticulum, and many mitotic figures (Fig. 3i), and variable amounts of HP-DU-1-positive cell surface markers (Fig. 3j). Seven % of explants showed only ductal hyperplasia, and 11% were necrotic.

Sections from MNUN-treated explants grown for 12 months or longer showed more variations in the amount of cell necrosis and proliferation. Malignant cells that reached explant surfaces showed a tendency to slough off and float in the medium. Central necrosis of explants was more common in explants cultured for 8 months or longer.

**In Vivo Growth**

**Control.** Cells from normal pancreas and control explants of 1, 2, 3, 4, and 6 months of culture failed to produce tumors in nude mice after 16 weeks. Sections from injection sites showed fibrosis and inflammation and rarely a recognizable ductule lined by degenerating epithelium surrounded by inflammatory infiltrates and foreign body giant cells.

**MNUN-treated.** Cells from MNUN-treated explants of up to 12 weeks formed small tumors at the site of injection but failed to grow beyond 4 weeks, gradually diminished in size, and disappeared. Sections from injection sites 16 weeks after inoculation showed fibrosis, inflammatory infiltrates, giant cells, and rarely a glandular structure formed by degenerating epithelium.

All 12 nude mice inoculated s.c. with 107 cells derived from 20- to 24-week-old MNUN-treated explants developed multiple s.c. nodules within 8 to 12 weeks. Tumor nodules varied greatly in size and number; histologically, all were consistent with adenocarcinoma (Fig. 4, a and b). Variations in the degree of tumor differentiation, inflammation, and necrosis were extensive. Severe necrosis in tumor nodules was noted in 6 mice. This was associated with fibroblastic proliferation and histiocytic infiltration of tumor tissues. In these cases, tumor proliferation at the periphery of the nodules was observed. Cystic degeneration of tumor nodules associated with hemorrhage occurred in 7 mice while other tumor nodules in the same mice showed solid non-cystic growth.

Tumor cells derived from nodules in mice were tumorigenic in 3 consecutive passages when injected s.c. Ultrastructurally, all tumor nodules were of epithelial origin; some were composed of cells forming glands with intraluminal microvillus projections (Fig. 4c), showing poorly developed rough endoplasmic reticulum, tonofilaments, and tonofibers (Fig. 4d), and expressing intense fluorescent staining with HP-DU-1 antibody (Fig. 4e).

Other tumors were composed of cells forming sheets with long interdigitating microvilli (Fig. 4, f and g), separated by irregular slit-like spaces well demarcated by HP-DU-1-positive tumor cells (Fig. 4h). No zymogen granules were noted in any of the tumor nodules, and only one of the tumor nodules examined showed periodic acid-Schiff- and mucicarmine-positive cells containing mucin granules (Fig. 4i).

This tumor was cystic and necrotic, and the tumor cells showed extensive variation in the expression of HP-DU-1 markers (Fig. 4j).

Sections from nude mice tumors stained with anti-keratin antibody showed some cells with a few to a moderate number of short and irregular fluorescent fibers which varied extensively in thickness. Both the cellular distribution, and the extent of variation in size and thickness of these fibers were similar to those of adult human pancreas ductular cells stained with this antibody.

**Karyotype analysis of primary outgrowths from 7 tumor nodules of nude mice revealed the tumor cells to be derived from human cells. The number of chromosomes in 83 to 90% of cells examined was 46; in about 8 to 10%, it was 45; and the remaining cells contained 42 to 44 chromosomes. Cells with endoduplicated chromosomes were occasionally seen. Cells with other chromosomal abnormalities were also observed (Fig. 5).**

**DISCUSSION**

There were great morphological variations between the pancreases of the same gestational age and some variations from day to day between and within the cultured explants from a given pancreas. The data, however, indicate that fetal human exocrine pancreas grows and differentiates fully to resemble adult morphology within 4 to 8 weeks in organ culture. In contrast with the classic concept, undifferentiated tubular structures did not express duct cell markers and therefore should not be considered as differentiated ducts or ductules. Acinar cell differentiation occurred 2 to 4 weeks before the appearance of duct cell antigens on the centroacinar and ductal cells.

Nitrosamines are effective carcinogens that produce tumors in many organs in a wide range of species. Their cytotoxicity, which may be important for the development of cancer, often overshadows their carcinogenic effects. In the present model, the rapid proliferation of the differentiating parenchymal cells appears to outweigh the cytotoxic effects of MNU. The cytotoxicity of MNU was confined to necrosis of certain cells, the loss of apical cytoplasm and zymogen granules associated with dilatation of acinar lumens, and the proliferation of cells with ductal antigen forming a core in the involved acinus. The proliferation of these cells may represent a nonspecific response of centroacinar, ductular, or dedifferentiated acinar cells to MNU.
Pancreatic carcinoma in humans usually occurs late in life and is uncommon before the fourth decade. During the latent period, progressive tissue and cellular changes are considered steps which eventually lead to the emergence of cancer cells. Repair deficiency and repeated and/or long-term exposure to these chemicals are likely to induce heritable lesions (initiated state) in target cells. A selective or promoting environment then allows the initiated cells to differentially produce focal growths which may evolve through preneoplastic and neoplastic stages to emerge as a malignant neoplasm. The selective persistence of $^{14}$C-methyl in target cells of pancreas explants was demonstrated after exposure to dimethylnitrosourea (12). The present data indicate the necessity of at least 3 months of exposure before the appearance of enhanced growth foci. While the length of exposure to carcinogen beyond 3 months required for the development of malignancy is yet to be determined, the continuation of MNU administration for an additional 8 to 10 weeks leads to the development of carcinoma. The longer latency period (more than 20 weeks) for the development of carcinoma in the fetal human pancreas explants with their relatively higher proliferative activity, compared with that of the adult human pancreas explants (10 to 12 weeks) (10) with little or no mitotic activity, suggests cumulative lifelong alterations in the adult pancreas induced by environmental chemicals which increase its susceptibility to MNU.

REFERENCES

Fig. 1. a, micrograph from 12-week fetal pancreas showing interconnecting tubules lined by low cuboidal epithelium surrounding an endocrine island and the formation of acinar structures (arrowhead). Bar, 50 μm × 250. b, micrograph from 12-week fetal pancreas cultured for 2 weeks, showing fluorescence of the acinar cells stained with AC1 antibody and fluorescein isothiocyanate anti-mouse IgG. Bar, 50 μm × 500. c, electron micrograph from 12-week fetal pancreas explant cultured for 3 weeks, showing well-developed acini with zymogen granules. Bar, 5 μm × 2,000. d and e, micrographs from 12-week fetal pancreases cultured for 4 weeks. Sections were stained with HP-DU-1 antibody showing centroacinar cells (arrowheads) (d) and duct cell surface fluorescence (e). Bar, 50 μm × 500. f and g, electron micrographs from 12-week fetal pancreases cultured in the absence of MNU for 10 and 12 months, respectively, showing necrosis, degeneration, and degranulation of acinar cells (f). A few cells are shown containing zymogen granules. Bar, 5 μm × 2,000. g, one of the few preserved acini containing zymogen granules. Bar, 5 μm × 4,000.
Fig. 2. a, electron micrograph from 12-week fetal pancreas cultured for 4 weeks and treated with MNU twice a week, showing individual cells with increased density (arrowheads). Bar, 5 μm. × 2,000. b and c, micrographs from explant similar to that in a showing acinar cell (a) as AC1-positive cells in b and HP-DU-1-positive cells in c. To be noted are cells in the acinar lining without either specificity (arrowhead). Bar, 50 μm. × 500. d and e, micrographs from 12-week fetal pancreas cultured for 5 weeks and treated with MNU showing AC1-positive cells (d) and a core of HP-DU-1-positive cells in the same “acinus” (e). Bar, 50 μm. × 500. f, micrograph from 12-week fetal explant cultured for 5.5 weeks showing numerous duct-like structures with large hyperchromic nuclei and angular projections (arrow) into the lumen. Bar, 50 μm. × 500. g, micrograph from explant similar to f showing duct-like structures lined by HP-DU-1-positive cells. Bar, 60 μm. × 400. h, electron micrograph from 12-week fetal pancreas cultured for 6 weeks and treated with MNU showing duct-like structures lined by cells devoid of zymogen granules. Occasional degenerating cells with increased density are seen (arrowheads). Acinar (a) and islet (i) cells with secretory granules are also seen. Bar, 5 μm. × 2,000.
Fig. 3. a, micrograph from 12-week fetal pancreas cultured for 10 weeks, MNU treated, showing papillary hyperplasia. Bar, 60 μm. x 400. b, electron micrograph from explant similar to that in a showing cells with relatively large nuclei, inconspicuous nucleoli, scanty cytoplasm, and no secretory granules. Bar, 5 μm. x 4,000. c, micrograph from explant similar to that in a, showing HP-DU-1-positive cells forming papillary projections. Bar, 70 μm. x 340. d, micrograph from 12-week fetal pancreas cultured for 12 weeks, MNU treated, showing hyperplastic glands with a growth focus (arrowhead). Bar, 60 μm. x 400. e, micrograph from 12-week fetal pancreas cultured for 20 weeks, MNU treated, showing irregular glands lined by atypical cells. Bar, 60 μm. x 250. f, electron micrograph from explant similar to that in e showing irregular glands formed by cells with large nuclei, multiple prominent nucleoli, and scanty cytoplasm without secretory granules. Bar, 5 μm. x 2,000. g, micrograph from explant similar to that in e showing highly fluorescent HP-DU-1-positive cells forming atypical glands. Bar, 50 μm. x 400. h, micrograph from 12-week fetal pancreas cultured for 24 weeks, MNU treated, showing irregular glands lined by malignant cells. Bar, 60 μm. x 400. i, electron micrograph from explant similar to that in h, showing cells with prominent nuclei, scanty cytoplasm, rare rough endoplasmic reticulum, and forming irregular glands. Bar, 5 μm. x 4,000. j, micrograph from explant similar to that in h, showing intense fluorescence of HP-DU-1-positive cells forming irregular glands. Bar, 50 μm. x 400.
Fig. 4.  a, micrograph from nude mouse 12 weeks after s.c. inoculation of cells derived from 24-week explant similar to that in b, showing adenocarcinoma and a mild inflammatory infiltrate. Bar, 50 μm × 160. b, same as a, showing glandular structures lined by malignant cells. Bar, 60 μm × 500. c, electron micrograph from a tumor in a nude mouse, showing cytoplasmic tonofilaments and tonofibers in cells with well-developed microvilli. Bar, 0.5 μm × 7,000. d, electron micrograph from tumor cells from a nude mouse, showing polysomes, mitochondria, and a few tonofibers. Bar, 1 μm × 10,000. e, micrograph from tumor in the same nude mouse as in d, showing HP-DU-1-positive cells forming irregular glandular structures. Bar, 5 μm × 200. f, micrograph from tumor cells in nude mouse showing portions of 3 cells with interdigitating microvilli and no lumen (arrowhead). Bar, 1 μm × 10,000. g, micrograph from tumor cells in nude mouse showing portions of 2 cells with scanty cytoplasm and interdigitating lateral surfaces (arrowhead). Bar, 1 μm × 10,000. h, micrograph from tumor in the same nude mouse as in g, showing sheets and nests of HP-DU-1-positive cells forming irregular slit-like lumens. Bar, 5 μm × 200. i, electron micrograph from tumor in a nude mouse showing mucus (m) granules. Bar, 1 μm × 25,000. j, micrograph from tumor in the same nude mouse as in i, showing HP-DU-1-positive cells forming irregular nests and glandular structures. Bar, 5 μm × 200.
Fig. 5. Tumor cell from a nude mouse showing a normal female human karyotype with an abnormality of chromosome 2.
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