Retention of Human Properties by a Xenografted Human Colonic Tumor, GW-77, Propagated in Unconditioned Hamsters[1] (35115)

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(Introduced by E. R. Fisher)

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GW-77 is a newly established transplantable neoplasm capable of expansive growth in the cheek pouch, intramuscularly, or subcutaneously in unconditioned, adult golden hamsters (1). Originating from a human tumor of the transverse colon having a carcinoidal pattern, GW-77 ultimately became exclusively composed of signet-ring, mucin-producing tumor cells consistent with adenocarcinoma (2). This unusual growth-behavior of a tumor of human origin in apparently normal xenogeneic hosts thus raises the question of its species-identity, which was dealt with in the present study by immunofluorescent and biochemical means.

Materials and Methods. Several GW-77 tumors growing in their 72nd and 80th passages (over 3 years) in the cheek pouches of unconditioned, adult golden hamsters (*Mesocricetus auratus*) were excised, washed several times in physiological saline and either prepared for immunofluorescent testing by directly staining 5-μ frozen sections with 1:4 dilution each of fluorescein-conjugated antihuman or antihamster serum, or homogenized in 0.25 *M* sucrose (1:10 dilution) by a motor-driven Potter-Elvehjem grinder. The resulting homogenate was centrifuged at 15,000*g* for 30 min at 4° and the supernatant was used for electrophoresis.

The isoenzymes of lactate dehydrogenase (LDH; EC 1.1.1.27) were demonstrated by disc electrophoresis (3), employing 5% acrylamide as resolving gel and 2% acrylamide in the spacer gel. One-tenth ml of a 1:5 dilution of the supernatant containing the enzyme was layered on top of the spacer gel by means of a tuberculin syringe. Bromphenol blue was also added to visualize the mobility front. After electrophoresis (Shandon apparatus) for about 1.5 hr in Tris–glycine buffer (pH 8.2), at a constant current of 3 mA/column, the gels were stained for approximately 15 min at 37° in the following medium: 4 ml of NAD (10 mg/ml), 4 ml of phenazine methosulfate (0.1 mg/ml), 2 ml of nitroblue tetrazolium (10 mg/ml), 6 ml of lithium lactate (1*M*), and 90 ml of Tris—phosphoric acid buffer, pH 8.6 (0.05 *M*).

Antisera to human and hamster antigens (neoplastic cell lines and tissue extracts) were prepared according to the method described by Stulberg *et al.* (4) in albino adult rabbits and conjugated with fluorescein isothiocyanate as described by Goldman (5). After absorbing twice with rabbit liver powder, the conjugates were cross-absorbed with human HeLa or hamster BHK-21 cells. Specificity was determined by a complete absence of staining with the heterologous cell suspensions. The stained sections were mounted with 50% glycerol and 50% phosphate-buffered saline (pH 7.2), and examined with a UV-fluorescent microscope.

Results. Whereas cryostat sections of GW-77 incubated with specific fluorescent antihamster serum did not show any peripheral fluorescence and only negligible and occasional total fluorescence, a majority of the cells reacting with antihuman serum principally showed a peripheral, surface, fluorescence (Fig. 1), indicating the presence of species-specific human antigen in the GW-77

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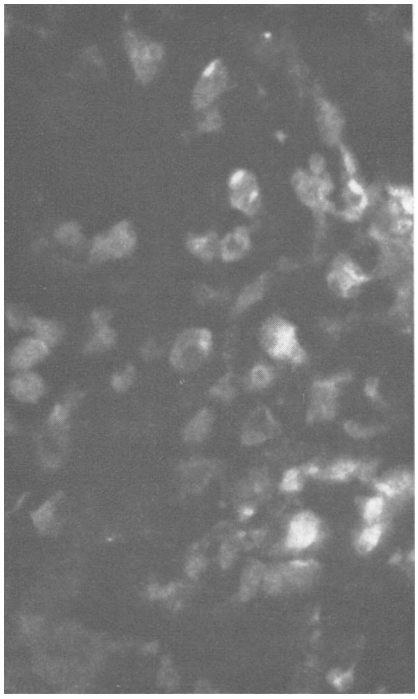

FIG. 1. Direct fluorescent antibody test of GW-77 tumor cells with fluorescein-conjugated antihuman rabbit serum, showing predominantly peripheral staining reaction; ×250.

tumor.

The bands resulting from our disc-electrophoretic separation of LDH have been redrawn in Fig. 2. GW-77 is here compared to the LDH isoenzyme profiles of the human HeLa-S3 cell line, harvested from monolayer culture, and the transplantable hamster amelanotic melanoma, A. Mel. No. 3, obtained from cheek pouch grafts and prepared as were the GW-77 tumors. The four bands seen in HeLa apparently represent, in their order from the origin to the anode, LDH-4, 3,2, and 1; LDH-5 is not demonstrable by this technique, probably because of its lying at the cathodic side of the origin. GW-77's LDH isoenzyme-mobility pattern exactly resembles that of the human HeLa cell line. The hamster's LDH isoenzyme profile, represented here by the A. Mel. No. 3 cell line, is, on the basis of the localization and number of its bands, the subband appearance, and the quantitative relationship between the heavier LDH-5 and LDH-4 isoenzymes, quite distinct from that of HeLa and GW-77. By this method, we almost always find 4 (3 prominent and 1 weaker) bands for LDH in human cells (either taken from tissue culture or surgical specimens) and 5 isoenzymes in hamster organs or *in vitro* cell lines. In terms of its LDH isoenzyme profile, the GW-77 tumor, even after being removed from the hamster cheek pouch, is unequivocally of the human type.

Discussion. The serial propagation of tumors in unconditional alien animals without rejection mechanisms destroying the xenografts, as in a number of our human tumor lines, naturally invites doubt as to their still being "foreign" to their new host species. Indeed, those human tumors that we have found to become regularly invasive and metastatic after transplantation to apparently normal hamsters do seem to have properties common with both their original and their animal host, such as species-antigen, karyotype, and LDH and glucose-6-phosphate dehydrogenase isoenzyme-mobility patterns (6), causing us to entertain the hypothesis that either human–hamster hybrids or chimeras, more likely the former, resulted (6, 7). In the case of the GW-77 tumor, which in the hamster morphologically resembles a mucin-producing signet-ring-cell adenocarcinoma, the retention of human properties, based upon species-specific surface antigen and LDH isoenzyme-mobility pattern, is consistent with its less malignant, only locally expansive, growth pattern in the hamster. Upon comparison of the malignant behavior of such human tumor xenografts to species-identity, it appears that degree of malignancy is related to degree of similarity of the xenograft to the new host species. It is tempting to speculate that this principle might also govern the behavior of neoplasms in the syn-

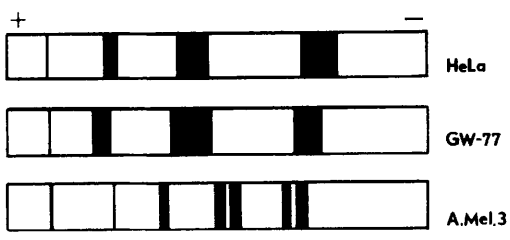

FIG. 2. Comparison of LDH isoenzyme-mobility patterns of HeLa, GW-77 and A. Mel. No. 3 tumors.

geneic situation.

Why a truly xenogeneic tumor such as GW-77 can grow without being rejected in its new host species, regardless of transplantation site, is not clear. In reporting a similar occurrence for a long-propagated human tumor which likewise produces mucin, Yohn *et al.* (8) suggested that the mucin covering the cells possibly protects them from the action of cytotoxic antibodies. It is therefore of interest to note that a third human tumor capable of growing in unconditioned hamsters, *GW-39*, likewise is mucin-producing (1, 9).

Summary. The unlimited serial propagation of a human colonic tumor, GW-77, in unconditioned, adult golden hamsters raises the question of its still possessing "human" properties. Both the direct fluorescent antibody test and its LDH isoenzyme-mobility pattern reveal the human species-specific character of GW-77. The retainment of its original species-identity is consistent with its relatively less malignant, only locally expansive, growth-behavior in various sites of the hamster.

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