Light Microscopic Observations of Transplantable Mouse Hepatomas

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SUMMARY

The origin, histology, generation time, and karyotype of four long-term, stable, transplantable mouse hepatomas are reviewed. All arose as well-differentiated, trabecular, hepatocellular carcinomas and each differentiated in a unique way. Hepatomas BH3 and BNL were induced in BUB mice and have been maintained by serial intrasplenic transplantation since 1959 for 229 and 452 generations, respectively. Hepatomas SS1G and SS1H are sublines of a hepatoma that arose spontaneously in a C3H/StWi mouse in 1949, and they have been maintained by serial s.c. transplantation for 277 and 107 generations, respectively. Hepatoma BNL has a diploid karyotype, hepatoma SS1G is polyploid, and hepatomas BH3 and SS1H each contain two cell lines, one diploid and one polyploid. Neither ploidy nor number and kind of marker chromosomes can be correlated with microscopic characteristics of the hepatomas.

MATERIALS AND METHODS

Two inbred strains of mice are used. The C3H/StWi strain is characterized by development of spontaneous hepatomas in about 40% of the males over 14 months of age. The BUB strain has a low incidence of spontaneous hepatomas; 2 were found in 138 mice examined at 14 months of age.

The hepatomas in C3H/StWi mice have been maintained by serial s.c. transplantation by injection of minced tumor into the axilla, and those in the BUB mice have been maintained by serial intrasplenic transplantation. Injection of tumor cells into the spleen can force some of them into the liver where secondary nodules also grow well (8). Hosts are 1.5 to 3 months of age and of either sex. The tumors are transplanted when they attain 0.5 to 1 cm in diameter.

A sample of donor tissue is fixed each generation in Bouin’s fluid for hematoxylin and eosin staining of paraffin sections to monitor the histology of the tumor. Occasional samples were prepared for routine histochemical identification of glycogen and lipid. The electron microscopy and correlated enzymic cytochemistry and biochemistry of the BUB tumors are described in separate papers (12, 13).

Karyotypes of the tumors were obtained by a modification of the procedure of Bunker (3). Tumor-bearing hosts were given injections of colchicine, 2 mg/kg, 3 to 4 hr prior to sacrifice, and the tumor cells were exposed to hypotonic sodium citrate, fixed in methanol:glacial acetic acid (3:1), air dried, and stained with Giemsa stain. A minimum of 35 metaphase figures were obtained for each tumor line. These were compared with karyotypes of adult hepatocytes undergoing mitosis after partial hepatectomy.

RESULTS

Hepatoma BH3 was induced in a BUB mouse fed for 9 months on a bentonite-containing diet (23) which renders mice choline deficient. The s.c. implants did not survive beyond the 1st generation, but intrasplenic ones grew readily. Generation time decreased gradually (Table 1) and stabilized at 2 weeks. The tumor is now in its 229th transplant generation. The cells of the primary liver tumor (Fig. 1) resembled normal hepatocytes with respect to nucleocytoplasmic ratio, presence of bile canaliculi, and organization into 1-cell-thick trabeculae. Nuclear and cell size diminished in the 1st transplant generation, and since then the histological appearance of the tumor has remained...
relatively unchanged (Fig. 2). After growth had stabilized, chromosome analysis of Generations 114 to 158 revealed 2 populations of cells, 1 diploid with 40 acrocentric chromosomes and 1 hypotetraploid with a mode at 68 chromosomes (Table 2). When transplanted s.c., small metastatic nodules appear in the lungs (13).

Hepatoma BNL was induced in a BUB mouse by injection of fragments of normal, mitotically inactive liver from a 90-day-old donor into the spleen of a young host (10). Subsequent intrasplenic transplants grew with increasing rapidity, and generation time soon stabilized at 1 week (Table 1). The tumor is now in its 452nd generation. The major change visible by light microscopy in the hepatocyte-like cells from the primary tumor (Fig. 3) has been a gradual diminution in the amount of cytoplasm (Fig. 4). Karyotyping between Generations 261 and 327 revealed a single diploid cell line (Table 2). Small nodules of these cells are found in the lungs, indicating that this tumor can metastasize (13).

Hepatoma SS1G arose as a spontaneous primary liver tumor in a 679-day-old male C3H/StWi mouse. Its slow growth during early generations (Table 1) changed abruptly in Transplant 52, and it then stabilized at its current generation time of 2 weeks. It is now in its 277th generation.

DISCUSSION

These transplantable mouse hepatomas are unusual because of their long history, ranging from 107 to 452 transplant generations, their retention of some structural characteristics of hepatocytes, and their current and long-term stable growth characteristics. All of the primary hepatomas from which they arose may be classified according to Reuber (18) as highly differentiated hepatocellular carcinomas or according to Stewart and Snell (20) as well-differentiated trabecular carcinomas. Unlike rat hepatomas (14, 18), edematous and follicular orientations of the cells did not appear during subsequent transplantations, and no duct-like patterns emerged in spite of the fact that under some physiological conditions nonneoplastic hepatocytes can revert to the ductular pattern of embryonic liver (9). All have retained their trabecular organization. If they are transplanted systematically at the times indicated in Table 1, there is no necrosis. The structural features characterizing them as hepatocellular include typical vesicular nuclei, bile canaliculi, and glycogen, visible by both light and electron microscopy (13, 22), and typical mitochondria, Golgi complexes, and endoplasmic reticulum detectable only in ultrastructural studies.

The histological appearance of each primary tumor (Figs. 1, 3 and 5), characteristic intermediate stage (e.g., Figs. 6 and 9), and recent transplants (Figs. 2, 4, 7 and 10) is unique. This is particularly well demonstrated by hepatomas SS1G and SS1H which were derived from the same original primary tumor and maintained under identical conditions. Similarly, Malick (12, 13) has shown that hepatomas BH3 and BNL are unique ultrastructurally al-
though each retains some hepatocyte features. For example, both retained typical nuclei, mitochondria, lysosomes, glycogen, and bile canaliculi and both acquired hypertrophied nuclei, retained fewer cell junctions, and lost all peroxisomes. However, rough- and smooth-surfaced endoplasmic reticulum and microvilli are abundant in BH3 and sparse in BNL. The review by Malick (13) revealed no correlation between ultrastructural features and growth rate, and we see none in relation to histological organization. This is somewhat at variance with the observations of rat hepatomas of different growth rate by Hruban et al. (7), but they note many exceptions to their general rule that the more rapidly growing tumors are the least differentiated.

The karyotype of normal BUB mouse hepatocytes in mitosis after partial hepatectomy was found to be chiefly diploid (40 chromosomes) and tetraploid plus some octaploid. Swartz (21) reports 36% diploidy, 56% tetraploidy, and 6.8% of higher ploidy in adult mice. Chromosomes are present in the diploid number in 3 of the 4 hepatomas described here (BH3, BNL, SS1H) and in polyplody numbers in 3 strains (BH3, SS1G, SS1H). The latter are aneuploid and exhibit a variety of marker chromosomes (Table 2). Because normal mouse chromosomes are similar in size and form, minor karyotypic changes might not be detected by the procedure used here. Furthermore, because a large proportion of normal hepatocytes are polyploid, it is possible that polyploidy in these tumors has little genetic significance. In rat hepatomas, Nowell et al. (17) were unable to find a correlation between diploidy and minimum deviation with respect to enzyme patterns, and more recently Wu and Morris (24, 25) reported a lack of correlation between chromosome number and the growth rate, the degree of differentiation, and enzymic activity. In this study we also have found no correlation between gross changes in the number of chromosomes or in the appearance of abnormal marker chromosomes and the structural and, in some tumors, ultrastructural and cytochemical changes in the tumor cells. It appears that the neoplastic variations depend on more subtle genetic changes than those expressed by whole chromosomes.

REFERENCES

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Fig. 1. Hepatoma BH3. The primary tumor induced in a BUB mouse fed a 50:50 bentonite:basal diet. The cells resemble normal hepatocytes. H & E, x 400.
Fig. 2. Hepatoma BH3. Transplant Generation 200 reveals trabecular organization, diminished cell size, and relatively normal nucleocytoplasmic ratio. H & E, x 400.
Fig. 3. Hepatoma BNL. Hepatocyte-like cells in a hepatoma induced by implanting normal liver cells by intrasplenic injection. H & E, x 400.
Fig. 4. Hepatoma BNL. Transplant Generation 430. Cytoplasm is greatly reduced in amount. H & E, x 400.
Fig. 5. Hepatoma SS1. The primary spontaneous tumor in a C3H/StWi mouse which gave rise to hepatomas SS1G and SS1H. H & E, x 400.
Fig. 6. Hepatoma SS1G. Transplant Generation 10. Large hepatocyte-like cells are distended by large vacuoles. Frozen sections stained with Sudan dyes revealed the contents of the vacuoles to be lipids. H & E, x 400.
Fig. 7. Hepatoma SS1G. Transplant Generation 268. Cytoplasmic vacuoles have disappeared and cell size is greatly diminished. Trabecular organization persists. H & E, x 400.
Fig. 8. Hepatoma SS1G. Karyotype of transplant Generation 198 reveals a hypetetraploid cell containing 88 chromosomes with 8 marker chromosomes represented by 4 pairs of apparently homologous chromosomes (top row) including 1 pair of minute chromosomes (far right). H & E, x 400.
Fig. 9. Hepatoma SS1H. Transplant Generation 44. Cells vary considerably in size but all bear resemblance to hepatocytes. H & E, x 400.
Fig. 10. Hepatoma SS1H. Transplant Generation 102. Compare with Fig. 5, the primary tumor; histological progression has been slight in 14 years. H & E, x 400.
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