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.

INTRODUCTION

Transplantable liver tumors of the rat have been used extensively in cancer research. Indeed, much of our knowledge concerning the deviations from normal in neoplasms is based on the remarkable series of rat hepatomas developed by Morris (14, 15) and Reuber (18). However, the use of more than 1 animal model is desirable to determine the extent of species variations and, thereby, the applicability of experimental results to the human disease. Unlike rat hepatomas, which may be hepatocellular, ductular, undifferentiated, or mixed tumors, hepatomas in mice are usually hepatocellular, although exceptions have been reported in 2 laboratories (16, 19). A few transplantable mouse hepatomas have been studied, but they were examined after a relatively small number of serial transplantations (1, 2, 4–6, 11). This paper describes the light microscopy of 4 transplantable hepatomas that have been maintained for 14 to 24 years and that, at this writing, have reached 107 to 452 transplant generations.

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relatively unchanged (Fig. 2). After growth had stabilized, chromosome analysis of Generations 114 to 158 revealed 2 populations of cells, 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. Throughout its early (Fig. 9) and recent (Fig. 10) history, it has retained its histological resemblance to hepatic tissue. Generations 75 to 80 contained 2 populations of cells, 1 diploid and 1 hypohexaploid, with a mode of 106 chromosomes (Table 2).

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 nucleoli, 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 a 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 polyploid 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 hypertetraploid 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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