Renal Cell Carcinoma in the Wistar-Lewis Rat: A Model for Studying the Mechanisms of Cholesterol Acquisition by a Tumor in Vivo

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ABSTRACT

Renal adenocarcinoma implanted into isogenic Wistar-Lewis rats closely resembles human renal cancer. This paper characterizes the tumor's growth rate, metastatic potential, and its light and electron microscopic appearance. Additionally, for the first time, the pathways through which a tumor acquires the cholesterol needed for growth were quantitated in vivo. Two 1-mg pieces of renal carcinoma were implanted beneath the renal capsule of 80 Wistar-Lewis rats. Of the implanted tumors 95% "took" and grew rapidly, doubling every 2.6 days initially. Growth slowed, however, to a doubling time of 8.3 days by the fifth wk. Twenty rats underwent surgical resection of the primary tumor 5 wk after implantation. Of these, 85% subsequently developed lung metastases. Histologically, the tumor had a clear-cell appearance due to the presence of large vacuoles, some of which contained glycogen. The esterified cholesterol content of the tumor was 3-fold higher than normal kidney during the initial period of rapid tumor growth and increased to a 14-fold elevation by 12 wk. The normal kidney in vivo had a high rate of uptake of cholesterol carried in low density lipoproteins and a low rate of de novo sterol synthesis. In contrast, the renal carcinoma lost most of its low density lipoprotein uptake activity and, instead, acquired the cholesterol needed for growth by a 5-fold increase in the rate of de novo cholesterol synthesis. This model may prove valuable in both testing therapeutic strategies directed against human renal cancer and understanding the regulation of cholesterol homeostasis in a growing cancer.

INTRODUCTION

Human renal cancer is characterized biologically by a propensity for lung metastasis and biochemically by a peculiar derangement of lipid metabolism that results in an excessive accumulation of esterified cholesterol in the malignant cells. The pathophysiological link between these two observations is not known for human renal cancer, but in a variety of animal tumor models (1, 2), an altered cellular cholesterol content has been associated with the premalignant state (3), tumor growth (4), and even the metastatic potential of an established tumor (5). We report here a detailed biochemical and morphological analysis of a renal adenocarcinoma which originated in a Wistar-Lewis rat and was originally described by White et al. (6). This tumor proved to be remarkably similar to human renal cancer in that it arose spontaneously, is neither hormonally dependent nor virally contaminated, and predictably metastasizes to the lungs and kills the host. Thus, as an animal tumor model which closely mimics a human disease, it may prove more basic level as a model for studying the relationship between a tumor's growth and its metabolism of cholesterol.

A major advantage of this model with respect to metabolic studies is that the tumor is isogenic to the host (unlike, for example, a human tumor transplanted into a nude mouse). Thus, species-specific, ligand-receptor interactions between the tumor and the host can be accurately measured in vivo. A tumor potentially can acquire the cholesterol needed for growth through two tightly regulated pathways, de novo cholesterol synthesis and receptor-mediated endocytosis of circulating LDL-cholesterol. Techniques for measuring in vivo the absolute rates of both de novo cholesterol synthesis (7, 8) and receptor-dependent LDL uptake (9-11) have been developed and validated for a variety of organs. In this study, these techniques were applied to the Wistar-Lewis rat renal carcinoma and provided the first quantitative data on rates of cholesterol acquisition by a tumor in vivo. The dynamic measurements of cholesterol flux through these two pathways, along with the static measurements of the tumor's free and esterified cholesterol content, can be correlated with the tumor's growth. Thus, these measurements provide the first detailed quantitative analysis of the pathways through which a cancer, in situ, acquires the cholesterol needed for growth.

MATERIALS AND METHODS

In all cases, male Wistar-Lewis rats (Charles River Breeding Laboratories, Wilmington, MA), between 125 and 150 g (i.e., weanling), were used for implantation. This is a syngeneic line of rats developed at the aforementioned facility. The donor tumor was obtained from Dr. R. K. Babayan of Boston University where the tumor was originally isolated. Tumor transplantation was performed by excising the tumor specimen from the live donor and placing it in chilled outgrowth medium (Hanks' solution; Gibco Laboratories, Grand Island, NY). A Brinkmann microtome was used to cut the tumor in three dimensions to prepare hundreds of 1.0-mm3 pieces (weight, about 1 mg). Following ether anesthesia, the left flank of the recipient was incised and the kidney exposed. Using a × 25 operating microscope, a small incision was made in the renal capsule, and two pieces of tumor were introduced. The kidney was replaced, and the skin was closed with wound clips. No sutures were placed in the fascia. On average, 2 workers could implant 20 rats in 2 h using a single pool of tumor specimens.

Growth curves were obtained by killing rats 14, 21, 28, 35, and 90 days after implantation. The tumors were weighed, and gross inspection was made for metastatic disease.

In a separate study of metastatic disease, tumor was implanted, and after 5 wk, a transabdominal nephrectomy was performed to remove the tumor-bearing kidney. The rats were then maintained for an additional 8 wk, following which they were killed to evaluate the occurrence of macroscopic metastatic disease involving the lungs.

Several procedures were used for morphological studies (12, 13). Samples were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections were stained for general histology with hematoxylin-eosin, for iron using Perl's method, and for RNA using methyl green pyronin. Frozen sections of formalin-fixed tissue were stained with oil red-O for neutral lipids. Other samples were fixed in ethyl alcohol and embedded in paraffin. Sections of alcohol-fixed tissue were stained for carbohydrates with the periodic acid-Schiff method, with
and without prior digestion with diastase (12). Sections of fresh frozen tissue were stained by the Gomori method for acid phosphatase. Additional samples were cut into small blocks and fixed in 3% glutaraldehyde in phosphate buffer. The specimens were postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated through a graded series of alcohols, and embedded in Epon-Araldite. Thick sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate (13). Tumor tissue, both primary and metastatic, was assayed for free cholesterol and esterified cholesterol content after separation on silicic acid/Celite columns using the ferric chloride reaction (14).

Five wk after implantation, the absolute rates at which the renal carcinoma and the adjacent kidney, adrenal gland, liver, and muscle synthesize cholesterol were measured in vivo using [3H]water as a labeled precursor (7) with correction for transfer of newly synthesized cholesterol from the liver to the carcinoma and other organs (7, 8). In the same tissues the rate of total (receptor dependent and independent) LDL-cholesterol uptake was measured in vivo using a primed, continuous infusion of [14C]sucrose-labeled Wistar-Lewis rat LDL as the probe (9–11).

RESULTS

Overall, in 80 animals implanted with two 1.0-mg pieces of tumor beneath the renal capsule, the take-rate was 95%. Failure seemed most often due to loss of the implant from under the renal capsule with subsequent intra-abdominal tumor formation in some of the animals. The incidence of successful transplantation did not vary over the length of the implantation period (90 days).

Among the 20 animals nephrectomized 5 wk after implantation, 85% had macroscopic metastatic disease in the lungs 8 wk later. Metastases i.p. rarely occurred and were usually the result of either direct tumor spread or the dislocation of one of the implants. The rare instance of liver involvement was associated with tumor on the surface of the liver and did not emanate from an intraparenchymal site.

The primary renal carcinoma grew rapidly as shown in Table 1. By 14 days after implantation, the tumor had grown from an initial weight of 2 mg to 80 mg. This corresponded to a doubling time of only 2.6 days. Thereafter, the growth rate steadily slowed to a doubling time of about 8 days from the fifth wk onward. By 3 mo following implantation, the rats were moribund with a massive tumor load, and if left alone, they would invariably succumb to the cancer.

Because the human renal clear cell carcinoma is known to have markedly elevated cholesterol ester levels (15), the cholesterol metabolism of this rat renal carcinoma was studied in detail. As shown in Table 1, the free cholesterol content of the tumor (i.e., the unesterified cholesterol located primarily within the plasma membranes) did not vary with time. In contrast, the cholesterol ester content of the tumor increased from 0.20 mg/g at 2 wk following implantation to 0.84 mg/g at 3 mo. At no time, however, did the cholesterol ester content of the rat carcinoma approach the massive cholesterol ester overload seen in the human clear cell carcinoma (10–15 mg/g). In general, the malignant renal tissue had a cholesterol ester content which was at least 3-fold higher than that present in normal kidney, and the cholesterol ester content increased as the rate of tumor growth slowed.

The quantitative importance of the two pathways through which this tumor could have acquired both the sterol needed for growth as well as the excess cholesterol stored as esters is shown in Fig. 1. The normal kidney is both control rats and normal rat kidney only about one-fifth of the total LDL clearance. In the tumor, however, the clearance of the methyl human LDL receptor was measured (16). The clearance of methyl human LDL is through a receptor independent mechanism and in normal kidney is only about one-fifth of the total LDL clearance. In the tumor, however, the clearance of the methyl human

Table 1 Growth characteristics and the cholesterol and glycogen content of the Wistar-Lewis rat renal carcinoma

Two-mg implants of the tumor were placed under the renal capsule at Day 0. Groups of six animals were then killed at the times shown, the carcinoma was weighed, and the content of cholesterol and glycogen was determined. For comparison, samples of normal kidney tissues from the same animals were run in parallel.
LDL probe was identical to the clearance of the rat LDL probe (6 μl/h/g), indicating that all LDL uptake by the tumor was through a receptor independent process. Moreover, the clearance of the methyl human LDL probe was the same in the renal tumor as it was in normal kidney, indicating that a permeability barrier was not present. Thus, the marked decrease in total LDL uptake by the tumor was due entirely to loss of functional LDL receptors on the tumor cell’s surface.

On gross inspection, the tumors were beige-to-tan in appearance and fixed to the renal bed. In tumors larger than 5 g, there was usually a central area of necrosis or hemorrhage. The larger tumors (>25 g) often had multiple blood-filled cysts. While the smaller tumors appeared to be encapsulated, the larger tumors clearly invaded neighboring organs, the most common being the spleen.

On light microscopic examination, the renal neoplasms were composed of solid nests of cells (Fig. 2A). The nuclei of the cells were large and contained one or more large nucleoli. Scattered foci of necrosis were present. The neoplasm had a clear cell appearance, since many of the cells contained large vacuoles (Fig. 2A). However, the large vacuoles did not contain neutral lipid demonstrable by the oil red-O stain (Fig. 2B). The neoplastic cells contained abundant glycogen in the cytoplasm and in some of the large vacuoles as demonstrated by the periodic acid-Schiff stain (Fig. 2, C and D). On chemical analysis (Table 1) the tumor glycogen content was increased 14-fold compared with normal kidney. The cells gave a weakly positive stain for RNA (methyl green pyronin). The large vacuoles did not stain positively for hemosiderin or acid phosphatase. The necrotic foci in the neoplasms did stain positively for neutral lipid, hemosiderin, and acid phosphatase.

On electron microscopic examination, the neoplastic cells...
Fig. 3. Survey electron micrographs of the renal cell carcinoma are shown. In A, the cells have large nuclei and multiple vacuoles of various sizes and are connected by desmosomes. x 4,520. In B, the cytoplasm of one cell exhibits mitochondria, rough-surfaced endoplasmic reticulum, abundant glycogen granules, three small lipid droplets (L), and a large vacuole containing glycogen granules. x 12,800.

exhibited desmosomal junctions typical of epithelial cells (Fig. 3). The cells contained large nuclei, mitochondria, rough endoplasmic reticulum, abundant glycogen granules, a few small non-membrane-bound lipid droplets, and numerous large single-membrane-bound vacuoles (Fig. 3). Some of the vacuoles were electron lucent (Fig. 4A). Others contained glycogen granules and membranous material (Fig. 4B). No viral particles were identified.

The histochemical and electron microscopic findings suggested that the vacuoles contained watery fluid, with or without glycogen, and other material but no lipid. The findings were also consistent with possible formation of these vacuoles by processes such as heterophagocytosis and autophagocytosis.

DISCUSSION

The renal adenocarcinoma described here is a highly aggressive tumor of the kidney. It arose spontaneously and appears to be neither virally contaminated nor dependent on hormones for growth. It can be readily transplanted beneath the renal capsule of recipient rats with high efficiency. It closely resembles human renal cancer in several respects. (a) Like the human tumor, the histological appearance is of a clear-cell carcinoma containing large vacuoles. Human renal cell carcinomas are composed of variable proportions of clear and granular cells. The clear cells contain sparse organelles, abundant glycogen particles, and variable amounts of lipid deposits and endocytic vacuoles (17-19). The latter structures, which are particularly prominent in the Lewis rat renal cell carcinoma cells, probably develop in relationship to retention by the carcinoma cells of the fluid transport capability of renal tubular epithelial cells. The cytoplasm and some of the vacuoles of the neoplastic cells stain positively for glycogen, which is consistent with the 14-fold elevation in glycogen levels found in the tumor compared to normal kidney. Thus, the human renal clear cell carcinoma...
and the Lewis rat renal cell carcinoma share many ultrastructural features.

(b) The tumor resembles human cancer in its biological behavior. If the tumor is implanted beneath the renal capsule, lung metastasis develops within 90 days in 85% of the recipients, even if the primary tumor has been resected. This predictability of metastasis, along with the ease and efficiency of implantation, makes this model useful for testing adjuvant therapy aimed at metastatic disease.

(c) This tumor is similar to human renal cancer in its metabolism of cholesterol, albeit with some important differences. The human clear cell renal carcinoma is massively overloaded with esterified cholesterol having levels as high as 15 mg/g (15), which is consistent with the 2-fold increase in ACAT activity in the tumor compared with normal kidney that has been reported (20). The Wistar-Lewis rat renal cancer reported here has a similar elevation in ACAT activity and a cholesterol ester level from 3- to 14-fold higher than the concentration present in normal kidney (0.06 ± 0.01 mg/g), but still less than the levels found in the human cancer. One possible explanation for the lower cholesterol concentration in the rat tumor is its rapid rate of growth when compared to the human neoplasm. The rat tumor initially doubles in size every 2 days and maintains a doubling time of only 8 days up to the time the animal dies; in contrast, the primary human tumor has a doubling time of 240–600 days (21). Based on the growth rate observed between the fourth and fifth wk following implantation (growth rate constant = 0.0035/h), the absolute mass of cholesterol required by the tumor simply to sustain growth can be calculated to equal 9.1 μg/h/g. This figure can then be compared to the 5.1 μg/h/g of cholesterol entering the tumor through the combination of synthesis and LDL-cholesterol uptake (Fig. 1). From this calculation it is apparent that the tumor needs all of the cholesterol it synthesizes and the small amount it takes up via LDL transport simply to create new cell membranes and grow. Furthermore, the tumor must also be taking up cholesterol from a source other than LDL (perhaps by uptake of cholesterol from HDL). This quantitative analysis of the cholesterol fluxes into the cancer may explain why the rapidly growing rat carcinoma did not accumulate large absolute amounts of cholesterol esters. The slowly dividing human clear cell carcinoma, on the other hand, with its much slower rate of new membrane formation, accumulates a massive excess of cholesterol esters.

In summary, the Wistar-Lewis rat renal cell carcinoma provides the investigator with a model that in many respects is similar to human renal cancer. This cancer arose spontaneously, is not hormonally dependent, is apparently free of virus, and metastasizes predictably from the kidney to the lung causing the animal’s death. Histologically, the tumor appears similar to human clear cell cancer. Biochemically, the animal tumor accumulates an excess of esterified cholesterol, but not to the same extent as the human tumor does. Quantitative analysis of the pathways through which the tumor acquires cholesterol showed a 5-fold increase in the rate of cholesterol synthesis but, unexpectedly, complete loss of receptor-dependent endocytosis of LDL-cholesterol. This animal tumor model may be particularly valuable for testing therapeutic strategies directed against human renal cancer and for determining why or how LDL receptor activity is lost during the malignant transformation process.

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REFERENCES

5. Rivnay, B., Gorelik, E., Segal, S., and Shinitzky, J. Plasma membrane
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