Low density lipoprotein-receptor activity is lost in vivo in malignantly transformed renal tissue

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Mammalian cells can acquire cholesterol through two tightly regulated pathways, namely de novo cholesterol synthesis and receptor-mediated endocytosis of circulating low density lipoprotein (LDL). Malignant cells growing in vitro acquire cholesterol through both mechanisms but the quantitative importance of these pathways to a cancer growing in vivo is not known. Using the Lewis rat renal carcinoma model, this study measured the rate of cholesterol acquisition via both pathways in vivo in both normal and malignant renal tissue. In contrast to normal kidney, after malignant transformation, LDL-receptor activity disappeared entirely and the cancer acquired the cholesterol needed for growth by a 5-fold increase in the rate of cholesterol synthesis.

LDL receptor *Cholesterol synthesis* *Malignant transformation* *Renal carcinoma*

1. INTRODUCTION

Cholesterol is a necessary constituent of eucaryotic cell membranes and consequently elaborate regulatory mechanisms have evolved to ensure an adequate supply of sterol for new membrane synthesis [1]. A mammalian cell can potentially acquire cholesterol through 3 pathways [2]. First, it can synthesize new cholesterol from acetyl-CoA via a tightly regulated pathway in which the formation of mevalonic acid is the rate-limiting step. Second, it can take up cholesterol from circulating LDL through a receptor-mediated endocytotic process (receptor-dependent LDL uptake) that, in cultured cells at least, is regulated at the level of transcription of the gene for the LDL receptor [3]. Finally, the tissue can acquire cholesterol from circulating LDL via a non-saturable, apparently unregulated, uptake process that does not depend upon specific LDL receptors (receptor-independent LDL uptake) [4]. In the live animal, the cells of even mature organs require at least some new sterol for maintenance of membranes and cell repair. After malignant transformation even greater amounts of cholesterol may be required to support cell proliferation and growth of the tumor. Presumably such malignant cells could acquire cholesterol through any (or all) of these 3 pathways. Up to now, however, there have been no quantitative data available regarding the importance (or even the existence) of receptor-dependent LDL uptake in malignant tissues in vivo. This study, therefore, measured the absolute rates at which both normal and malignantly transformed renal tissue acquire cholesterol through each of these 3 pathways under in vivo conditions. The results show that normal kidney in the rat acquires cholesterol predominantly through receptor-dependent LDL uptake and has a low rate of sterol synthesis. After malignant transformation, however, LDL-receptor activity disappears entirely and the cancer acquires the

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Abbreviations: LDL, low density lipoprotein. WHHL, Watanabe heritable hyperlipidemic

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cholesterol needed for growth by a 5-fold increase in the rate of cholesterol synthesis.

2. MATERIALS AND METHODS

The Wistar-Lewis rat renal adenocarcinoma [5] used in this study offered the major advantage of being homologous to the host (unlike, e.g., a human tumor transplanted into a nude mouse). Therefore, species specific ligand-receptor interactions such as receptor-dependent LDL uptake could be accurately quantitated. Tumor tissue was obtained from a single tumor-bearing rat and cut into pieces weighing approx. 1 mg. Two of these pieces were then microsurgically implanted beneath the renal capsule of 80 syngeneic Lewis recipient rats. After 5 weeks the tumor-bearing rats were divided into 2 groups. In one group, the rates of LDL clearance by both the renal tumor and the contralateral normal kidney, as well as the liver, were measured using a primed-continuous infusion of either [^{14}C]sucrose-labelled rat LDL [6,7] (total LDL clearance) or [^{14}C]sucrose-labelled methyl-human LDL (receptor-independent LDL clearance) [8]. With this method, the plasma specific activity of the labelled LDL is held constant for the 6 h infusion period so the label accumulates in tissues as a linear function of time. Thus, the tissue clearance (μl/h per g) of LDL for all tissues can be measured and when multiplied by the plasma LDL concentration, the absolute rate of LDL-cholesterol entry into each tissue is obtained (μg/h per g). The rat LDL probe measures total LDL clearance whereas the methylated-human LDL probe, which does not interact with the rat LDL receptor [7] measures receptor-independent LDL clearance. The second group was injected with 100 mCi [^{3}H]water and killed 1 h later to measure absolute rates of cholesterol synthesis in the renal tumor, normal kidney and liver [9,10].

3. RESULTS

3.1. *Tumor growth and metabolic effects*

Throughout the 5 week period, the recipients appeared healthy and none died. From a weight of 2 mg at implantation, the tumors grew to a mean weight of 1590 ± 160 mg by 5 weeks. Only 2 of the 80 recipients failed to show tumor growth. To exclude the possibility that the presence of the tumor might indirectly alter, e.g. by suppressing food consumption, either the expression of LDL receptors or the rate of cholesterol synthesis in other tissues, the rates of total LDL uptake and cholesterol synthesis were measured in the liver and kidney of tumor-bearing rats and in sham-operated control rats. It was found that the presence of the tumor did not affect either process. Thus, a direct comparison of LDL uptake and cholesterol synthesis could be made between normal and malignantly transformed renal tissue in vivo in the same animal.

3.2. *Receptor-independent and receptor-dependent LDL transport by the tumor*

Total LDL clearance in the normal kidney occurred, as reported [4], at one-third the rate observed in the liver (table 1). The renal tumor, however, cleared LDL at only one-fifth the rate found in the normal kidney. Importantly, receptor-independent LDL clearance was essentially the same in the kidney and the renal tumor (table 1,

Table 1

Comparison of 3 pathways through which normal renal tissue, malignant renal tissue and liver acquire cholesterol

Tissue	LDL clearance (μl/h per g)			Absolute mass of cholesterol acquired via each pathway (μl/h per g)		
	Total clearance	Receptor-independent clearance	Receptor-dependent clearance	Receptor-independent LDL transport	Receptor-dependent LDL transport	De novo synthesis
Normal renal	31 ± 1	5 ± 1	26 ± 1	0.7 ± 0.1	4 ± 0.1	0.9 ± 0.1
Malignant renal	6 ± 1	6 ± 1	0	1.0 ± 0.1	0	4.1 ± 0.4
Liver	93 ± 4	8 ± 1	85 ± 4	1.2 ± 0.1	12.8 ± 0.6	48.4 ± 3.9

column 2); thus, the decrease in total LDL clearance seen in the tumor resulted from complete loss of receptor-dependent LDL uptake by the malignant tissue (table 1, column 3). This loss is seen even more dramatically when these clearance values are expressed as absolute rates of LDL-cholesterol uptake and compared directly to the rates of total and receptor-independent LDL transport observed in the normal kidney of non-tumor-bearing control rats (fig.1). As is apparent, the data obtained in the kidneys of tumor-bearing animals are superimposable upon the standard kinetic curves for LDL transport in the kidney, i.e. the rate of receptor-independent LDL-cholesterol uptake equaled 0.7 ± 0.1 μg/h per g while receptor-dependent uptake equaled 4.0 ± 0.1 μg/h per g (table 1, columns 4 and 5). In contrast, the renal tumor manifested no receptor-dependent LDL transport so that the uptake of homologous LDL and methylated-human LDL were essentially equal and, furthermore, both of these uptake rates fell on the curve defining the rate of receptor-independent LDL transport in the normal kidney (fig.1).

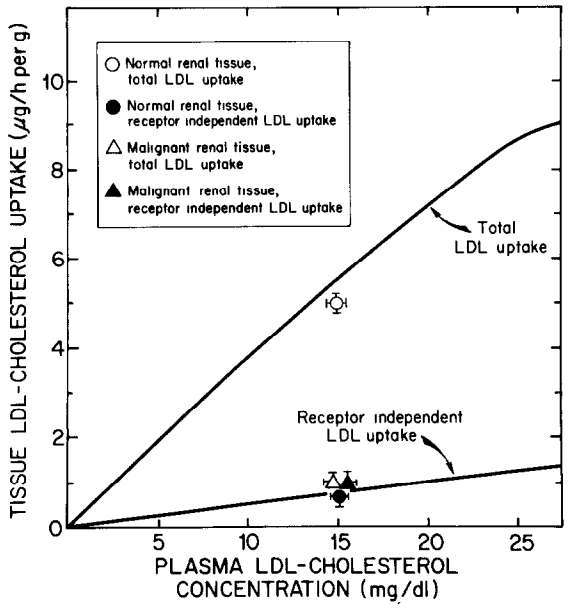

Fig.1. Total and receptor-independent LDL uptake in normal and malignant renal tissue. The kinetic curves show the rates of total and receptor-independent LDL-cholesterol uptake into the kidney as a function of the plasma LDL-cholesterol concentration in control rats without tumors. The lower curve, obtained using methylated-human LDL, illustrates that receptor-independent LDL uptake by the kidney is a linear function of the plasma LDL concentration. In contrast, total LDL transport (upper curve) in the normal kidney shows a curvilinear relationship to the plasma LDL-cholesterol concentration. The difference between these 2 curves represents the magnitude of the receptor-dependent component of LDL transport in the normal kidney at any concentration of plasma LDL-cholesterol. Superimposed upon these curves are the data obtained in the present study for homologous rat LDL uptake and methylated-human LDL uptake in both the kidney and malignant renal tumor obtained from the tumor-bearing animals.

3.3. *Cholesterol synthesis by the tumor*

As expected, the rates of incorporation of [^{3}H] water in vivo into cholesterol were high in the liver (1550 ± 125 nmol/h per g) and much lower in the kidney (30 ± 5 nmol/h per g); however, the incorporation rate in the renal tumor was nearly 5-fold higher (132 ± 12 nmol/h per g) than the kidney. When these incorporation rates were converted to absolute rates of sterol synthesis, the tumor was found to synthesize 4.1 ± 0.4 μg cholesterol/h per g while only 0.9 ± 0.1 μg/h per g were synthesized in the normal kidney. Thus, as summarized in columns 4–6 of table 1, with the loss of receptor-dependent LDL transport, most of the sterol acquired by the tumor cells came from de novo synthesis rather than from the uptake of LDL-cholesterol.

4. DISCUSSION

While many malignant cells adapted to grow in vitro express LDL-receptor activity [11–15], it has not been possible to measure in absolute terms whether this pathway is responsible for cholesterol acquisition in the malignant tumor in vivo. The data reported here were made possible by the development of techniques for quantitating the rates of receptor-dependent and receptor-independent LDL uptake and sterol synthesis under in vivo conditions [6–8] and clearly demonstrated that all receptor-dependent LDL-transport activity was lost after malignant transformation of the kidney. This is similar to the situation seen in the WHHL rabbit that genetically lacks receptor-dependent LDL transport [16]. In the kidney of normal rabbits the rates of homologous and methylated-

human LDL clearance equal 11 and 3 μl/h per g, respectively, while in the kidney of the WHHL rabbit both of these LDL preparations are cleared by the kidney at 3 μl/h per g [17]. Hence, the renal adenocarcinoma reported here in the rat behaves like the normal kidney of the receptor-deficient rabbit.

The complete loss of LDL-receptor activity in vivo after malignant transformation was unexpected and runs counter to what would have been predicted on the basis of a variety of malignant cells growing in vitro [11-15]. The fact that receptor-independent LDL transport was not affected by transformation means that the loss of receptor-dependent LDL transport was not due to a change in capillary permeability within the tumor. If this loss of receptor activity is found to be a general characteristic of malignant tissues in vivo, then the LDL-receptor mechanism cannot be utilized to deliver anti-tumor agents to primary or metastatic malignancies in vivo but, inhibition of cholesterol synthesis in the tumor coupled with a reduction in circulating plasma cholesterol levels could conceivably be utilized therapeutically to control tumor growth.

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REFERENCES

[1] Bloch, K. (1983) Crit. Rev. Biochem. 14, 47-92.
[2] Dietschy, J. (1984) Klin. Wochenschr. 62, 338-345.
[3] Brown, M., Kovanen, P. and Goldstein, J. (1981) Science 212, 628-635.
[4] Spady, D., Turley, S. and Dietschy, J. (1985) J. Clin. Invest. 76, 1113-1122.
[5] White, R. and Olsson, C. (1980) Invest. Urol. 17, 405-412.
[6] Pittman, R. and Steinberg, D. (1979) J. Biol. Chem. 254, 6876-6879.
[7] Spady, D., Bilheimer, D. and Dietschy, J. (1983) Proc. Natl. Acad. Sci. USA 80, 3499-3503.
[8] Spady, D. and Dietschy, J. (1985) Proc. Natl. Acad. Sci. USA 82, 4526-4530.
[9] Turley, S., Andersen, J. and Dietschy, J. (1981) J. Lipid Res. 22, 551-559.
[10] Dietschy, J. and Spady, D. (1984) Lipid Res. 25, 1469-1476.
[11] Faust, J., Goldstein, J. and Brown, M. (1977) J. Biol. Chem. 252, 4861-4871.
[12] Goldstein, J., Brown, M., Kreiger, M., Anderson, R. and Mintz, B. (1979) Proc. Natl. Acad. Sci. USA 76, 2843-2847.
[13] Barnard, G., Erickson, S. and Cooper, A. (1984) J. Clin. Invest. 74, 173-184.
[14] Vitols, S., Grahrton, G., Ost, A. and Peterson, C. (1984) Blood 63, 1186-1193.
[15] Ho, Y., Smith, R., Brown, M. and Goldstein, J. (1978) Blood 52, 1099-1114.
[16] Schneider, W., Brown, M. and Goldstein, J. (1983) Mol. Biol. Med. 1, 355-367.
[17] Spady, D. and Dietschy, J. (1985) submitted to J. Lipid Res.