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CORRELATION OF TUMOR METASTASIS WITH STEROL CARRIER PROTEIN AND PLASMA MEMBRANE STEROL LEVELS

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Summary - Squalene and sterol carrier protein (SCP) levels and sterol/phospholipid molar ratios of whole cells and plasma membranes were measured in cultured primary tumor and metastatic cell lines. SCP is abundant in all cell lines. However, metastatic lines have significantly lower SCP levels and plasma membrane sterol/phospholipid ratios than do primary lines. The results indicate that extremely malignant, metastatic cells are unable to produce or maintain adequate levels of both SCP and plasma membrane sterols when grown in lipoprotein deficient media. This defect, *in vivo*, probably causes excess uptake of SCP and lipid.

One generally accepted characteristic of the malignant process is a change in the normal regulation of lipid metabolism and transport, permitting formation of new membranes and cellular proliferation. Our previous studies focused on defects in regulation of cholesterol transport exhibited by malignant cell lines derived from human renal tumor metastases (e.g. (1-2)). These cells in culture have the same or markedly lower levels of cholesterol when compared to normal kidney cells in culture (3). However, *in vivo*, they cause tumors which accumulate cholesterol (1-4). Decreases or increases in the cholesterol content of these cells are paralleled by similar changes in the cellular level of squalene and sterol carrier protein (SCP) (1-2). SCP (also called

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fatty acid binding protein (5)) is considered to be a major regulator of lipid metabolism and transport (2,6). The purpose of the studies described here was to determine whether other types of cultured malignant cells, in particular highly metastatic cells, share properties of cultured renal cancer cells with regard to SCP and sterol levels.

Materials and Methods

Tissue Culture and Cell Lines - LM cells were obtained from the American Type Culture Collection (CCL 1.2) and grown in suspension in a chemically defined serum free medium (7). Cells were injected into C_3H/Hen (MTV-) or athymic (nude) mice (BALB/c, nu/nu) obtained from Charles River Laboratories, Wilmington, NY, and from Frederick Cancer Center, Frederick, MD. Primary tumor cells and lung metastasis cells were isolated and cultured in suspension in the above chemically defined serum-free medium (8-9). B-16 melanoma cell variants F10 and F1 were obtained through the generosity of Dr. E.M. Jensen, E.G. & G. Mason Research Institute, Worcester, MA, and Dr. I.J. Fidler, Frederick Cancer Research Center, Frederick, MD. The cells were cultured in monolayer with 10% heat inactivated fetal calf-serum (10,11).

Membrane Isolation and Sterol/Phospholipid Assay - Plasma membranes were prepared as described before (7,10). Plasma membranes from all cells were enriched 6-8 fold in (Na^+,K^+)-ATPase activity as compared to the corresponding crude homogenate. The sterol normally found in LM fibroblasts, C_3H primary cells, C_3H metastatic lines, Nu primary cells, and Nu metastatic lines (when the cells are cultured in the absence of serum) is desmosterol. Desmosterol/phospholipid ratios and cholesterol/phospholipid ratios (in B-16 melanoma variants) were assayed by previous methods (7).

Squalene and Sterol Carrier Protein (SCP) Assay - SCP was quantitated from whole cell homogenates by immunoprecipitation (6).

Results and Discussion

SCP Levels and Desmosterol/Phospholipid Ratios in LM Fibroblast Derivative Cells - Total SCP levels and desmosterol/phospholipid ratios of whole cells and plasma membranes were quantitated in C_3H primary tumor and metastatic cell lines. The cells were cultured in suspension under identical conditions and in the absence of serum. The data in Table I show that SCP is a remarkably abundant component in these tumor cells, i.e., 20-30 μg/mg total protein. Furthermore, the level of SCP in C_3H

Table I
Plasma Membrane Desmosterol/Phospholipid Ratios and SCP Levels of Cultured Primary Tumors and Lung Metastases from C_3H Mice[a]

Cell Line	SCP (mg/100 mg protein)	Desmosterol/Phospholipid	
		Whole Cell	Plasma Membrane
C_3H Primary Cell Line	3.00 ± 0.01	0.20 ± 0.03	0.40 ± 0.02
C_3H Metastasis 1.1	2.07 ± 0.05**	0.27 ± 0.05	0.24 ± 0.06*
C_3H Metastasis 1.2	2.20 ± 0.01**	0.28 ± 0.06	0.32 ± 0.02*
C_3H Metastasis 2.1	2.03 ± 0.03**	0.21 ± 0.05	0.21 ± 0.04**
C_3H Metastasis 2.2	2.10 ± 0.01**	0.13 ± 0.03	0.17 ± 0.04**
C_3H Metastasis 2.3	2.17 ± 0.01**	0.18 ± 0.03	0.14 ± 0.03**
Mean of all Metastases	2.11 ± 0.03***	0.21 ± 0.03	0.22 ± 0.03***

[a]Primary tumor cells and lung metastatic cell lines were obtained from C_3H mice and cultured under identical conditions in serum free chemically defined medium as described in Materials and Methods. SCP and desmosterol/phospholipid molar ratios were determined in whole cells and isolated plasma membranes of cultured cells also as described in Materials and Methods. Values represent the mean ± SEM (n=3-5). A single, double, and triple asterisk signifies $p < 0.05$, $p < 0.01$ and $p < 0.001$ by Students T test, respectively, as compared to the C_3H primary tumor cell line.

metastatic cell lines is significantly lower than in the primary cell line (Table I). Concomitantly, the plasma membrane desmosterol/phospholipid ratio is also significantly less (Table I). However, there is no change in whole cell sterol/phospholipid ratios of either primary or metastatic cells. When metastatic cell lines were grown in the presence of serum, the levels of both SCP and membrane sterol approached those of primary cells. Desmosterol was also replaced by cholesterol. The latter findings indicate metastatic cells are more dependent on external sources for lipid and, probably, SCP than primary cells.

Since injection of LM fibroblasts into C_3H/Hen mice may give rise to a highly selected C_3H primary cell population, LM cells were also injected into immunodeficient athymic nude mice. The properties of LM and other cells apparently do not change when injected into nude mice and subsequently returned to culture (e.g. (9,12)). Furthermore, the Nu primary cell line is identical to

Table II

Plasma Membrane Desmosterol/Phospholipid Ratio of Cultured Primary Tumors and Lung Metastases from Athymic (Nude) Mice[a]

Cell Line	Desmosterol/Phospholipid	
	Whole Cell	Plasma Membranes
Nu Primary Cell Line	0.21±0.03	0.50±0.05
Nu Metastasis 0	0.10±0.06	0.21±0.01***
Nu Metastasis 1	0.20±0.02	0.29±0.04*
Nu Metastasis 2	0.22±0.04	0.27±0.05*
Mean of all Metastases Line	0.20±0.01	0.25±0.02***

[a]Primary tumor cells and lung metastatic cell lines were obtained from athymic (nude) mice and cultured under identical conditions in a serum-free chemically defined medium as described in Materials and Methods. Values represent the mean ± SEM (n=3-10). A single and triple asterisk signify $p < 0.05$ and $p < 0.001$, respectively, by Students T test, as compared to the Nu primary cell line.

the original LM fibroblast cell line with regard to lipid composition and membrane properties (9). Similar to findings with C_3H mice (Table I), plasma membranes of the metastatic cell lines derived from lung metastases of nude mice have significantly lower desmosterol/phospholipid ratios when compared to ratios for the primary cell line (Table II). Another potentially important observation is that plasma membrane sterol/phopholipid ratios of cultured primary tumor cells are 2.0-fold (Table I) and 2.4-fold (Table II) higher in comparison to whole cell ratios. However, there is no apparent difference in the plasma membrane and whole cell ratios of metastatic cell lines; cf. Tables I and II.

SCP and Cholesterol/Phospholipid Ratio of B-16 Melanoma Variant Cells - Similar to LM cells (Table I), SCP is abundant in melanoma cells (15-20 μg/mg total protein). Again, SCP levels in the highly metastatic B-16-F10 melanoma cell line are strikingly lower than those of the less metastatic B-16-F1 melanoma line cultured under identical conditions (Table III). Similarly, the plasma membrane cholesterol/phopholipid ratio of the highly

Table III

SCP and Cholesterol/Phospholipid Ratios In B-16 Melanoma Variants[a]

Cell Line	Phenotype	SCP	Cholesterol/Phospholipid	
		(mg/100 mg)	Whole Cell	Plasma Membrane
B-16-F1	Low Metastatic	1.93 ± 0.03	0.40 ± 0.03	0.66 ± 0.04
B-16-F10	High Metastatic	1.47 ± 0.03**	0.21 ± 0.02**	0.24 ± 0.10**

[a] B-16 melanoma variants were cultured as described in Materials and Methods. Values represent the mean ± SEM (n=3) and a double asterisk signifies $p < 0.01$ by Students T test, as compared to the B-16-F1 line.

metastatic cell line is markedly lower than that of the less metastatic line. However, in contrast to findings with LM metastatic cell lines (Tables I and II), the whole cell sterol/phospholipid ratio of highly metastatic melanoma cells is also low when compared to the whole cell ratio of the less metastatic cells (Table III). These differences may reflect cell culture conditions, i.e., the absence of serum in the LM cell media and the presence of fetal calf serum in the melanoma cell media. They indicate that the highly metastatic melanoma cells are even more dependent on external sources of lipid than LM metastatic cells. As seen with LM primary tumor cells, the plasma membrane cholesterol/phospholipid ratio of less metastatic B-16-F1 cells is higher in comparison to the whole cell ratio (Table III). Also, there are no differences in these values for the highly metastatic B-16-F10 cells, similar to LM metastatic cell lines; cf. Tables I, II and III.

In these studies we concentrated on the abundant, ubiquitous protein called SCP (2,6). The results show that SCP is also abundant in highly malignant LM and melanoma cells. However, the level of SCP in these cultured cells is inversely related to the degree of malignancy or metastatic ability. Furthermore, the

cellular level of SCP correlates directly with the plasma membrane/phospholipid ratio, which also decreases with increasing metastatic ability. This close correlation between SCP and sterol levels is in accord with our previous findings in human renal cancer cells and fibroblasts and rat adrenal cells (1-2). Also, many tissues are able to accumulate SCP from serum, where it is associated with the lipoprotein fraction (13). Related reports by others are that low levels of SCP and lipid occur in rapidly growing hepatomas (14), and a decreased cholesterol/phospholipid ratio is a property of leukemic lymphocytes (15) and lymphotoxin resistant L cell populations (16).

Thus, it appears that, in culture, most highly malignant cells will exhibit lower levels of SCP and membrane sterol than primary or normal cells. However, past experience with various human tumors suggests that, *in vivo*, most tumors will contain higher than normal levels of SCP (1-2). Therefore, it is likely that during development of malignancy, a cell loses its ability to produce or maintain normal levels of SCP and lipids. Cell surface receptors may become modified so that SCP and lipid are rapidly taken up from serum. This defect becomes apparent, *in vitro*, when highly malignant cells are grown in lipoprotein deficient media and leads to unregulated cell division and proliferation, *in vivo*, in the presence of adequate blood (lipoprotein) supply.

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