Expression of the M_r 67,000 Laminin Receptor Is an Adverse Prognostic Indicator in Human Thyroid Cancer: An Immunohistochemical Study[1]

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ABSTRACT

Increased expression of the M_r 67,000 laminin receptor (LR) is a consistent event which appears as cancer cells acquire an invasive and metastatic phenotype. The M_r 67,000 LR is one of the many laminin-binding proteins able to interact with the major glycoprotein of basement membranes, laminin.

The recent development of a specific monoclonal antibody directed against the M_r 67,000 LR MLuC5 has allowed us to study large retrospective groups of human cancers with the aim of correlating the M_r 67,000 LR expression to the clinical, pathological, and survival data of the patients. A significant correlation has already been established between the increased expression of M_r 67,000 LR and survival of patients with breast, colon, ovary, lung, and endometrial cancers. In this study, we investigated the possibility that the detection of M_r 67,000 LR in thyroid human cancers could also be of prognostic value. We analyzed the expression of M_r 67,000 LR with immunohistochemistry using MLuC5 antibodies in paraffin sections of 40 benign and 170 malignant thyroid human tumors. We found that M_r 67,000 LR was not usually detectable in normal thyroid tissues adjacent to the lesion. Only 3 of the 40 thyroid adenomas examined (7.5%) presented cells positive for M_r 67,000 LR. For the malignant thyroid tumors examined, we found that 22.3% of papillary thyroid carcinomas, 38% of follicular thyroid carcinomas, 40% of poorly differentiated carcinomas, 25% of medullary carcinomas, and 58.3% of anaplastic carcinomas expressed a high level of M_r 67,000 LR. Although no correlation between the M_r 67,000 LR expression and survival was found in patients with follicular thyroid carcinomas, papillary thyroid carcinomas, anaplastic carcinomas, and medullary carcinomas, there was a significant correlation in primary thyroid cancers. Our data represent the first extensive study of the M_r 67,000 LR expression in human thyroid cancers and strongly suggest that its detection could be of prognostic value in the investigation of primary thyroid cancers.

INTRODUCTION

Human thyroid cancer is the most frequent malignant lesion occurring in the endocrine system. It accounts for 1.1% of all human cancers in the United States. Since this malignant disease usually afflicts young adults, it raises even more concern than its incidence might suggest. The principle histological subtypes of thyroid cancers are papillary, follicular, medullary, and undifferentiated; undifferentiated or anaplastic carcinomas are the most aggressive tumors and are typically present at an advanced stage. The prognosis of thyroid cancers is directly dependent on the invasive and metastatic capability of the cancer cells. Because it is usually difficult to distinguish between benign and malignant thyroid tumors, with the exception of papillary carcinomas, the development of new prognostic and diagnostic indicators is needed. Such markers could be developed from our understanding of the molecular mechanisms which are involved during tumor invasion and metastasis. Tumor progression results from complex successive interactions between cancer cells and old tissues (1). Invasion of basement membranes is a crucial step of tumor invasion and metastasis. During this process, interaction between malignant cells and the basement membrane glycoprotein laminin appears to play a key role (2–5). Cancer cells attach to laminin through a variety of cell surface molecules (6, 7). Among the 17 laminin-binding proteins identified, the M_r 67,000 LR[3] has been extensively studied in malignancy (1), and it is the first LR identified. It binds laminin with high affinity and has been found to be overexpressed in cancer cells with high invasive and metastatic potential. A correlation between M_r 67,000 LR expression and poor prognosis has been established for breast (8, 9), colon (10, 11), lung (12), ovary (13), cervical (14), and gastric cancers (15). The recent development of a monoclonal antibody (16) which recognizes, in particular, the M_r 67,000 LR on paraffin sections has allowed us to perform large retrospective studies of archival material. When we started to perform our studies, there were no data in the literature

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[3] The abbreviations used are: LR, laminin receptor; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; PDC, poorly differentiated carcinoma; MC, medullary carcinoma; AC, anaplastic carcinoma.

Table 1 M_r 67,000 LR expression in 40 benign tumors and 130 malignant thyroid tumors

Tumors	No. of cases	Age, yr (mean)	Sex		M_r 67,000 LR expression	
			Male	Female	No. of positive cases	%
Adenomi	40	46	8	32	3[a]	7.5
PTC	85	46	30	55	19	22.3
FTC	21	51	10	11	8	38.0
PDC	20	36	5	15	8	40.0
AC	24	66	7	17	14	58.3
MC	20	54	7	13	5	25.0

[a] $P = 0.047$ (Pearson's χ^2 test).

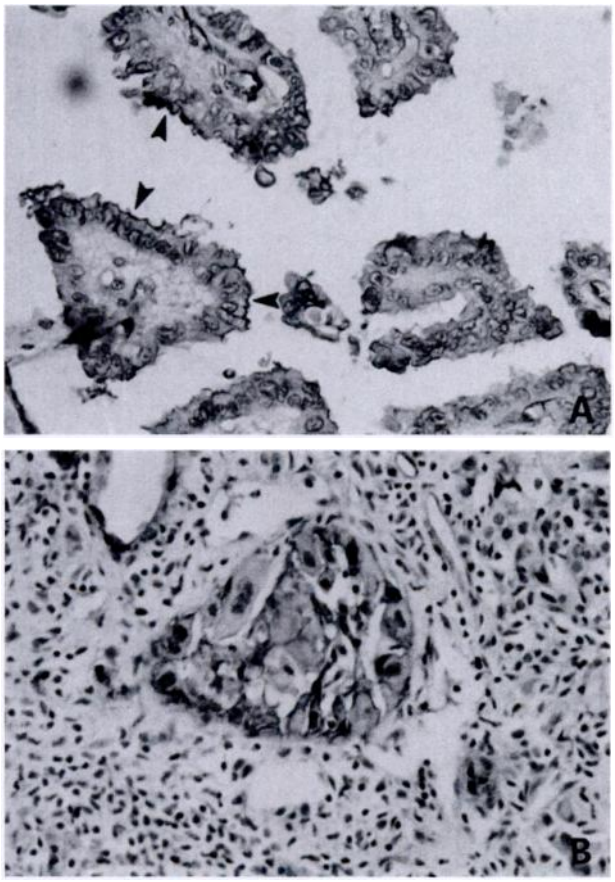

Fig. 1 M_r 67,000 LR immunostaining in thyroid cancers. *A*, cellular membrane of neoplastic cells showed immunoreactivity for M_r 67,000 LR. *B*, expression of M_r 67,000 LR in an undifferentiated carcinoma. *A* and *B*, ×300.

regarding the M_r 67,000 LR expression in human thyroid carcinomas. Therefore, we began a survey of the M_r 67,000 LR expression using the monoclonal antibody MLuC5 on a collection of benign and malignant lesions of the thyroid. We found that the M_r 67,000 LR expression increases in the more aggressive forms of thyroid cancers. Furthermore, we observed a significant association between survival and the M_r 67,000 LR expression in papillary thyroid carcinomas.

MATERIALS AND METHODS

Patients and Follow-Up. The study was carried out on 170 patients with primary malignant thyroid tumors and on 40 patients with benign neoplasms. This series of thyroid tumors is part of a larger series of thyroid cancer patients followed at the Institute of Pathology, University of Pisa. We studied all patients who received primary surgical treatment at the University of Pisa and whose tissues were available at the Department of Pathology. All patients with PTC, FTC, PDC, and MC were treated by total thyroidectomy followed by radioactive iodine at the University of Pisa. Patients with AC were treated with total thyroidectomy whenever possible, followed by external radiotherapy and/or chemotherapy. All patients were regularly followed up through physical examination, chest roentgenogram, and whole-body scan with ^{131}I (differentiated thyroid cancer). We studied patients with PTC, FTC, and PDC with at least 5 years of follow-up, whereas the minimum follow-up was 2 years for AC.

Immunohistochemistry. One hundred seventy malignant thyroid tumors (85 PTCs, 21 FTCs, 20 PDCs, 24 ACs, and 20 MCs) and 40 benign tumors (micro-macrofollicular adenomas) were collected from the files of the Institute of Pathology, University of Pisa. Immediately after surgery, the tissues were fixed in 10% formalin, embedded in paraffin, and stained with H&E.

LR Expression. LR immunostaining was performed on paraffin sections using immunohistochemistry. The avidin-biotin-peroxidase complex method was used as described previously (17). Briefly, the sections that had been previously deparaffinized and treated with block endogenous peroxidase were incubated with biotinylated horse anti-mouse IgG for 30 min and were then incubated overnight with the primary antibody (MLuC5, 10 μg/ml). The sections were then incubated with a 1:200 dilution of biotin-labeled secondary antibody for 30 min and avidin-biotin-peroxidase (Vector, Burlingame, CA) for 45 min. Subsequently, sections were stained for 5 min with 0.05% 3,3-diaminobenzidine tetrahydrochloride, 0.01% H_2O_2 in 0.05 M Tris-HCl buffer (pH 7.6), counterstained with hematoxylin, dehydrated, and mounted.

Immunohistochemical Evaluation and Statistical Analysis. Each section was carefully examined for the presence of cell membrane immunostaining for LR. At least 1000 cells were counted for each case. The tumors were considered LR when at least 2% of the positive cells were reactive.

The STATISTICA (Stat-Soft) package was used for statis-

Table 2 Percentage of M_r 67,000 LR-positive cells in thyroid cancers

		% of LR-positive cells							
		0		1–20		21–50		>50	
Histotype	No. of cases	No.	%	No.	%	No.	%	No.	%
PTC	85	48	56.4	12	14.1	15	17.7	10	11.7
FTC	21	13	62	1	4.7	1	4.7	6	28
PDC[a]	20	12	60	0	0	5	25	3	15
AC[a]	24	10	41.6	0	0	2	8.3	12	50
MC	20	15	75	0	0	3	15	2	10

[a] $P = 0.01$ between papillary and anaplastic carcinomas (contingency tables).

Table 3 Correlation between LR expression and survival in thyroid tumors[a]

			LR expression		
Cases	No. of cases	Mean survival (mo)	No. of positive cases	%	*P*
All series					
Alive	131	74 ± 35	31	23.6	
					<0.0001
Dead	39	26 ± 51	23	58.9	
Tumors with a good prognosis (papillary, follicular, medullary carcinoma)					
Alive	115	74 ± 35	26	22.6	0.04
Dead	11	80 ± 100	6	54.5	
Different histotypes					
PTC					
Alive	83	86 ± 33	17	20.4	0.04
Dead	2	86 ± 42	2	100	
FTC					
Alive	16	74 ± 29	6	37.5	NS[b]
Dead	5	80.2 ± 17	2	40	
PDC					
Alive	16	100 ± 33	5	31.2	NS
Dead	4	31 ± 6	3	75	
MC					
Alive	16	38.7 ± 20	3	18.7	NS
Dead	4	40.5 ± 18	2	50	
AC					
Alive	0				
Dead	24	9.5 ± 6	14	58.3	

[a] Statistical analysis evaluated using contingency table (χ^2 with Fisher's correction).
[b] NS, not significant.

tical analysis, and the following tests were used: (*a*) Kruskal-Wallis ANOVA median test and (*b*) Fisher's exact test.

RESULTS

LR Expression in 170 Thyroid Carcinomas. To evaluate the M_r 67,000 LR expression during tumor progression, benign tumors and carcinomas with different degrees of malignancy, all derived from the thyroid, were tested using immunohistochemistry with a monoclonal antibody specifically directed against the receptor. As reported in Table 1, only 3 of 40 (7.5%) adenomas were found to immunoreact with the MLuC5 monoclonal antibody, and the percentage of positive cells in these three cases was less than 5%. On the contrary, malignant tumors showed a high percentage of positivity. MCs displayed a 25% positivity, with a high frequency of tumors expressing less than 50% of positive cells. PTCs, FTCs, and PDCs showed an overall positivity of 33.4%, the positive FTCs presenting a higher percentage of receptor-expressing cells than the tumors of the other two histotypes. A large number of ACs were found to be receptor positive, with a high frequency of positive cells (Fig. 1). All data are summarized in Tables 1 and 2.

Correlation between the M_r 67,000 LR Expression and Survival in 170 Thyroid Cancers. To investigate whether the M_r 67,000 LR could be associated with disease outcome, the patients were divided into two groups according to their status: alive or dead. As shown in Table 3, of the whole series, 23.6% of the tumors from patients still alive expressed the LR, whereas 58.9% of the tumors from patients who had died from the disease were found to be positive ($P < 0.0001$). When only tumors with a good prognosis (*i.e.*, PTCs, FTCs, and MCs) were considered, the difference in the M_r 67,000 LR expression remained evident; as a matter of fact, 22.6% of the tumors from patients still alive were positive *versus* 54.5% in the group with poor outcome ($P = 0.04$). Within the different histotypes, no

statistically significant differences were found since the subgroups were very small in number, but in all of the cases, tumors with a poor outcome were more frequently M_r 67,000 LR-positive than the tumors from patients still alive.

DISCUSSION

Thyroid cancers constitute a heterogeneous group of tumors with widely varying prognoses. In this study, we have examined the expression of the M_r 67,000 LR in a collection of 210 thyroid tumors according to the immunoperoxidase technique using the monoclonal antibody MLuC5. The LR, the first cell surface molecule identified as a laminin-binding protein, has been extensively studied in human malignancy (8–10). Increased expression of the M_r 67,000 LR, both at the protein and mRNA levels, has been demonstrated in experimental models (9, 11) and in a large variety of human cancer lesions (8–10). Increased expression of the M_r 67,000 LR is usually associated with a poor prognosis. This has been demonstrated for breast (8), ovary (13), colon (10), and gastric cancers (14), with the exception of cervical cancer where mRNA for LR is correlated with cell proliferation rather than with invasive characteristics of the cancer cells (15). Because there are several cell surface proteins able to bind laminin, the exact function of M_r 67,000 LR during tumor invasion has not been clearly defined. There is however evidence (18) showing that this receptor is involved in the motility response of cancer cells toward laminin and participates in the adhesion of circulating cancer cells to the endothelium. In agreement with the key role of M_r 67,000 LR or the position of the invasive phenotype, we have previously demonstrated (19) that this cell surface receptor is up-regulated in trophoblastic cells.

Prior to our studies, there were no substantial data regarding the M_r 67,000 LR expression in thyroid cancers. We found that the M_r 67,000 LR was detected in the most aggressive forms of thyroid cancers. In fact, we observed highly significant differences in survival patients based on detection or nondetection of the LR in their primary tumors: interestingly, undifferentiated carcinoma of the thyroid, which is the most aggressive form of thyroid cancer, showed the highest percentage of positive cancer cells. The observation that benign tumors do not generally exhibit detectable levels of M_r 67,000 LR supports the involvement of this receptor during invasion and metastasis of the thyroid cancer rather than in the proliferation of the tumor cells. Our study indicates that the detection of the M_r 67,000 LR in primary thyroid cancers could be of great prognostic value in patients with primary thyroid cancers. Extensive prospective studies should determine the detection of M_r 67,000 LR in thyroid cancer and should be part of the routine evaluation of primary thyroid cancers.

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