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Original Paper

Expression of the 67 kD Laminin Receptor in Human Ovarian Carcinomas as Defined by a Monoclonal Antibody, MLuC5

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Previous immunohistochemical data from our laboratory have demonstrated that expression of the 67 kD laminin receptor (67LR), a cancer-associated, high-affinity laminin-binding protein, is upregulated in ovarian carcinoma cells compared with normal serosal cells, and that this increased expression in cancer cells could be related to patient outcome. The aim of this study was to validate MLuC5, a monoclonal antibody that recognises the 67LR, as a tool to perform future immunohistochemical studies on larger populations of ovarian carcinoma patients. Expression of the 67LR was determined in 51 primary human ovarian carcinoma samples using immunohistochemistry and MLuC5. The 67LR was detected in ovarian carcinoma cell clusters of variable extent. Analysis of the data determined that 67LR expression was significantly increased in the samples from patients with disease progression, compared with those with no evidence of disease after completion of primary therapy, and in pooled grade 2 and 3 tumours compared to borderline and grade 1 tumours ($P<0.05$, chi-squared test). No other significant correlation between 67LR expression and other clinicopathological parameters could be established. These data suggest that the 67LR is correlated to ovarian tumour progression. Detection of the 67LR using this monoclonal antibody could constitute an interesting parameter in prognosis determination of ovarian cancer.

Key words: ovarian cancer, laminin receptor, immunohistochemistry
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INTRODUCTION

ADVANCED OVARIAN cancer is a common cause of mortality and morbidity in women [1]. Late diagnosis at an advanced stage of the disease is usually related to silent extension of the disease into the abdominopelvic cavity [2]. Thus, in order to understand better the biology of these tumours and adequately prescribe the available therapeutic strategies, it is necessary to find reliable markers for ovarian tumour aggressiveness.

Expression of the invasive and metastatic phenotype by cancer cells is associated with their ability to cross basement membranes that constitute natural barriers between tissues [3]. Adhesion of the cancer cells to laminin, a major component of the basement membranes, is a key event in tumour invasion [4]. Biochemical studies of this crucial step led to the observation that the multiple biological interactions between cancer cells and laminin are mediated through a variety of cell surface proteins able to bind to laminin [4]. The 67 kDa high-affinity laminin receptor (67LR) was the first molecule characterised as able to bind to laminin [5–7]. Experimental data have demonstrated its implication to tumour migration and attachment to laminin [8]. Interestingly, its expression is upregulated in a large variety of cancer types [4, 9]. We have recently demonstrated that 67LR expression in ovarian cancer is associated with tumour aggressiveness: indeed, immunohistochemical staining of ovarian cancer sections has shown that the 67LR is significantly overexpressed in cancer cells from patients whose cytoreductive surgery was suboptimal, and those with poor clinical outcome [10]. However, the availability of the rabbit polyclonal antiserum directed against a cDNA-derived peptide [11] used in the latter study is limited.

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A recently developed monoclonal antibody raised against small cell lung carcinoma cells and named MLuC5 has been found to recognise the 67LR [12]. Immunohistochemical evaluation of 67LR expression in 1160 breast cancer samples has demonstrated a clear prognostic significance of 67LR detection using MLuC5 [13].

We therefore decided to evaluate 67LR expression in a series of 51 human ovarian carcinoma samples by immunohistochemical staining using the MLuC5 monoclonal antibody, and to evaluate the prognostic significance of this parameter by comparing 67LR expression with the clinicopathological characteristics of the tumours.

MATERIALS AND METHODS

Tissue samples

Slides from paraffin-embedded primary epithelial ovarian carcinoma samples were obtained from Ospedale San Gerardo, Monza and Clinica Valduce, Como, Italy. Clinical data and pathological reports, including histological type and differentiation grade, were available for each patient. Out of the 51 tumours, there were 41 serous, 2 mucinous, 3 endometrioid, 4 undifferentiated and 1 clear cell carcinoma. Surgical staging was established according to the FIGO (Fédération Internationale de Gynécologie-Obstétrique) [14]. Five tumours were classified stage I, 4 were stage II, 36 were stage III and six were stage IV. Four tumours were borderline, 6 were grade 1, 13 were grade 2 and 28 were grade 3 [15, 16]. All patients completed primary treatment including, if necessary, first-line chemotherapy after surgery. Debulking status was defined according to the size of the nodules left in the peritoneal cavity after surgery (no residual tumour after surgery, 8 patients; <2 cm, 9 patients; 2–5 cm, 9 patients; 5–10 cm, 8 patients; >10 cm, 15 patients; data not available, 2 patients). Follow-up status was derived from clinical and, if appropriate, radiological and surgical data (second-look), and was expressed as: NED, no evidence of disease; AWD, alive with disease; DOD, dead of the disease. AWD or DOD defines progression of the disease after primary therapy, and did not necessarily correlate with response to therapy defined during second-look evaluation. The follow-up ranged from 5 to 94 months (median, 36 months).

Immunoperoxidase staining

Detection of the 67LR in the ovarian carcinoma slides was performed by immunohistochemistry using the ABC Vectastain Elite kit (Vector Laboratories, Burlingame, California, U.S.A.) according to the supplier protocol. Briefly, tissue sections were deparaffinised in xylene and rehydrated in phosphate buffered saline (10 mM sodium phosphate, 0.9% NaCl, pH 7.5). Blocking of the endogenous peroxidase was performed with 0.3% hydrogen peroxide in methanol and the non-specific serum-binding sites were blocked with normal horse serum (1:20, Vector). The MLuC5 antibody diluted 1:500 in PBS was incubated for 2 h at room temperature. The slides were then incubated with biotinylated horse antimouse antibody (1:200) followed by exposure to preformed streptavidin-biotinylated horseradish peroxidase complex. Peroxidase was revealed by the 3,3′ diaminobenzidine tetrahydrochloride reaction [17]. Finally, sections were counterstained with haematoxylin, dehydrated and mounted. Controls included omission of the MLuC5 antibody and for a positive control, a breast cancer specimen was routinely used. Two independent observers estimated the level of MLuC5 staining by counting the cancer cells that were strongly stained by the antibody, expressed as a percentage of the total number of cells examined (at least 1000 cells per specimen). Staining was evaluated as follows: 0, no staining; 1, 0–10% of the cells were positive; 2, 10–50% of the cells were positive; 3, 50–100% of the cells were positive for MLuC5 immunostaining.

Statistical analysis

The means of the two immunostaining evaluations were used in the statistical tests. The differences between ovarian carcinoma specimens grouped according to the available clinicopathological data were determined by the chi-squared test.

RESULTS

Expression of the 67LR in ovarian carcinoma samples

The slides were immunostained with the MLuC5 monoclonal antibody using the avidin–biotin technique (Figure 1). Ovarian adenocarcinoma cells were heterogeneously stained with patchy positivity throughout the tumour cells areas, and values ranged from 0 to 3. This heterogeneous positivity could be observed in single (×200) microscopic fields and was found to be constant throughout the tumour. Cancer cells were either very strongly stained, or not stained at all by the monoclonal antibody (Figure 1). Staining was both cytoplasmic and plasma membrane-associated. Positivity of the cells was semiquantitatively evaluated according to the method described in Materials and Methods: 14 tumours were not stained by the antibody; 15 were 1+, 14 were 2+ and 8 were 3+. The stroma was always negative for 67LR immunostaining. In contrast, endothelial cells were always strongly stained by the antibody (Figure 1c).

Increased 67LR expression correlates with progression of the disease and tumour differentiation

Statistical evaluation of MLuC5 staining values according to the clinicopathological characteristics of the tumours was performed using the chi-squared test. Tumours from patients presenting with subsequent progression of the disease (alive with disease, AWD, or dead of the disease, DOD) were characterised by significantly more tumour positivity for MLuC5 immunostaining, defined by positivity of more than 10% of the cancer cells (i.e. graded 2 or 3) than tumours with no subsequent evidence of the disease (NED) (18/34, 53% versus 4/17, 23%, $P<0.05$, Table 1 and Figure 2). When considering tumour grade with the same criteria for staining positivity, pooled grade 2 (G2) and grade 3 (G3) tumours presented with increased frequency of MLuC5 positivity compared with borderline (BL) and grade 1 (G1) tumours (21/41, 51% versus 1/10, 10%, $P<0.05$, Table 1 and Figure 2). When considering the response to chemotherapy, there was a trend that was not statistically significant between the patients who showed complete or partial remission (60.6% of these tumours were MLuC5 negative), and those which did not respond (stable or progression, 36.4% were MLuC5 negative).

Finally, patients were grouped according to histological type, surgical stage and residual tumour after primary debulking surgery, but statistical analysis of MLuC5 staining in these groups of patients did not reveal statistically significant differences (Table 1).

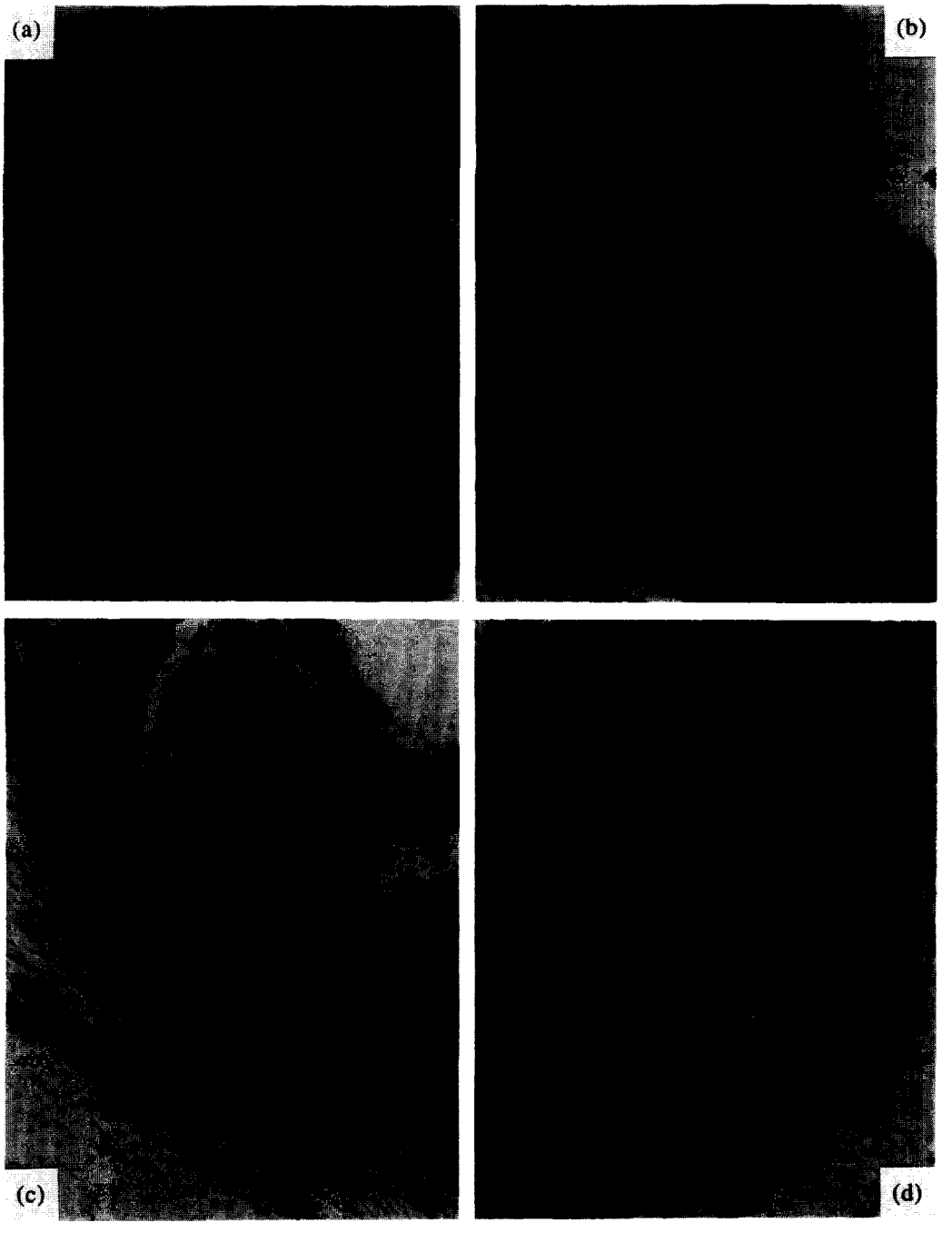

Figure 1. Typical examples of MLuC5 immunostaining of ovarian carcinoma samples. (a) Ovarian adenocarcinoma cells with no detectable MLuC5 immunoreactivity (score 0); capillary endothelial cells are positive. (b) Ovarian carcinoma cells displaying high expression of the 67LR, with clear membrane and cytoplasmic pattern (score 3). (c) Example of an islet of strongly positive adenocarcinoma cells (score 1). (d) Tumour sample with 60% MLuC5 positive cells (score 3) (original magnifications, ×400, except D, ×200).

DISCUSSION

Development of specific markers of ovarian cancer prognosis is a major challenge in gynaecological cancer research. Indeed, the biological aggressiveness of epithelial ovarian tumours is variable from tumour to tumour, and incompletely predicted by classical prognosis markers such as tumour stage, histological grade and size of the residual tumour [1, 2]. Thus, to improve the adaptation of the intensity of the therapeutic strategies to a given ovarian carcinoma, it is mandatory to develop new strategies for effective evaluation of the prognosis of this type of tumour. Biochemical studies of the mechanisms allowing cancer cells to invade host tissues and to disseminate have led to the identification of several gene products that could play a role during tumour progression [3]. The 67LR, a high-affinity laminin-binding protein, is thought to be involved in tumour cell attachment and migration to laminin, a major basement membrane glycoprotein [8], and its increased expression in carcinoma cells is usually related to prognosis [4, 9]. Detection of the 67LR in tumour cells could constitute the basis for new prognosis evaluation strategies. We previously demonstrated that, as in a variety of human carcinomas such as breast, colon and gastric carcinomas

Table 1. Correlation between MLuC5 positivity in the ovarian carcinoma cells and the clinicopathological characteristics of the tumours

Parameter		MLuC5 staining Negative (<10%)	Positive (>10%)
Histological type	Serous	23	18
	Mucinous	2	0
	Endometrioid	1	2
	Undifferentiated	3	1
	Clear cell	0	1
Stage	I	3	2
	II	3	1
	III	21	15
	IV	3	3
Grade	Borderline	4	0
	G1	5	1
	G2	5	7
	G3	15	14
Residual tumour*	Absent	4	4
	<2 cm	7	2
	2–5 cm	3	6
	5–10 cm	7	1
	>10 cm	7	8
Follow-up	NED	13	4
	AWD	4	3
	DOD	12	15
Response to chemotherapy†	Complete remission	10	5
	Partial remission	10	8
	Stable	1	5
	Progression	3	2

Positivity of each ovarian tumour for MLuC5 immunostaining is defined by strong staining of more than 10% of the cancer cells analysed (scores 2 and 3, see Materials and Methods).
The values correspond to the number of patients in the group.
NED, no evidence of disease; AWD, alive with disease; DOD, dead of the disease; G1, grade 1; G2, grade 2; G3, grade 3.
*Data were not available for 2 patients. †7 patients (6 stage I and 1 stage II–borderline) were not given chemotherapy.

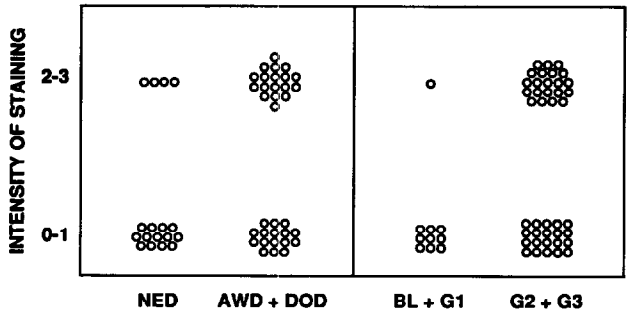

Figure 2. Expression of the 67LR detected by immunohistochemistry using MLuC5 according to follow-up and tumour grading. Intensity of staining was defined as described in Materials and Methods. NED, no evidence of disease; AWD, alive with disease; DOD, dead of the disease; BL, borderline; G1, grade 1; G2, grade 2; G3, grade 3.

[4, 9], ovarian cancer cells overexpress the 67LR, and that this overexpression is correlated to disease progression after primary therapy [10]. However, due to the lack of availability of the polyclonal antisera used, this study could not be expanded to larger patient populations. The development of a monoclonal antibody, MLuC5, that recognises the 67LR and provides strong prognosis predictive value in breast cancer, could help to circumvent this problem [12, 13].

In this study of 51 ovarian carcinomas, we demonstrate that 67LR immmunostaining of the ovarian cancer cells from the primary tumours using MLuC5 is correlated with patient follow-up and with tumour grading. Although not statistically significant, there was also a trend between MLuC5 immunostaining and response to chemotherapy. As suggested in previous studies [10], our data constitute an additional argument for the involvement of the 67LR, a high-affinity cell surface laminin-binding protein, in ovarian cancer progression.

The heterogeneity observed in MLuC5 immunostaining of otherwise phenotypically identical ovarian cancer cells further illustrates the classical concept of tumour cell heterogeneity [18–20]. Increased 67LR expression by cancer cells could correspond to a specific step in the acquisition of the invasive phenotype.

In conclusion, this study confirms 67LR detection in cancer cells from the primary tumour as an interesting parameter in prognosis determination for ovarian cancer, and establishes the equivalence of MLuC5, a monoclonal antibody that recognises the 67LR, to previously used polyclonal antisera. Future larger prospective studies are needed to validate 67LR detection in ovarian cancer for routine prognosis evaluation.

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