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INVERSE EXPRESSION OF TWO LAMININ BINDING PROTEINS, 67LR AND GALECTIN-3, CORRELATES WITH THE INVASIVE PHENOTYPE OF TROPHOBLASTIC TISSUE+

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Tumor invasion of host tissues and trophoblastic penetration of the endometrium share common biological features. Both processes involve the invasion of basement membranes, an event that is initiated by adhesion of cancer or trophoblast cells to basement membrane components and particularly to laminin. Adhesion to this latter glycoprotein is mediated through a variety of cell surface receptors. We have previously shown that the 67 kD Laminin Receptor (67LR) and a 31 kD Human Laminin Binding Protein, recently renamed galectin-3, are inversely modulated as the invasive phenotype of cancer cells progresses, with up regulation of the former, and down regulation of the latter, respectively. In this study, we examined the expression of these two proteins in 27 human trophoblastic specimens at different gestational ages using Northern and Western blot techniques. Expression of the 67LR increased from 7 weeks to a maximum at 12 weeks, when invasion is maximal, and then decreased. Expression of galectin-3 was inversely modulated by the gestational age, with a minimum expression at 12 weeks. Our data demonstrate that invasive trophoblast displays the same pattern of laminin binding proteins expression than invasive cancer cells, and further demonstrates that invasion of the extracellular matrix by trophoblast and cancer cells share common molecular mechanisms.

Invasion of the endometrium by trophoblastic cells shares several similarities with cancer invasion of the extracellular matrix. Indeed, after hatching from the zona

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pellucida, the blastocyst attach to the uterine epithelium, and invades the endometrium and its adjacent basement membrane. This invasion process is spatially limited to the endometrium: it stops when the trophoblastic cells have penetrated the uterine arterioles (1). Similarly, during their progression, cancer cells cross the basement membranes several times in order to invade the host tissues, during intra- and extravasation, and during invasion of muscle and nerves (2). This phenomenon is uncontrolled, continuous and not limited, whereas trophoblastic invasion takes place only during the first trimester of pregnancy, with maximal invasion at 12 weeks.

The penetration of basement membrane by cancer cells is a complex phenomenon that necessitates the coordinated expression of many proteins that allow the invading cells to attach, degrate, and migrate (2).

Degradation of the natural barrier formed by the basement membranes is mediated through the secretion of proteases such as the 92 kD and the 72 kD type IV collagenases, two members of the metalloproteinase family. It has been previously shown that the two enzymes are upregulated in trophoblastic cells in a pattern similar to the one found in invasive cancer cells (3, 4).

Adhesion of cells to laminin, a major basement membrane glycoprotein, is a key step that initiate this process (2). The 67 kD laminin receptor (67LR) is a high-affinity cell surface protein with high affinity for laminin. It has been shown to play a major role during tumor cell migration (5). Its expression is increased in cancer cells, and correlates with the invasive phenotype (6). Galectin-3 is a lectin that we identified in melanoma cells as a laminin binding protein initially named HLBP31 (7). Galectin-3 binds to the poly-N-acetyllactosamine residues of laminin (8). Its expression is decreased, and is inversely correlated with invasiveness of tumors, such as colon (7) and breast (manuscript in preparation). The up and down regulation of the 67LR and galectin-3, respectively, appears to be associated to the invasive phenotype, allowing the cancer cell to attach and detach from laminin as the invasion process progresses.

In this study, we examined the expression of the 67LR and galectin-3 in trophoblastic tissues at different ages of gestation, corresponding to various invasive phenotype, in order to further document the similarity between trophoblast and cancer invasion.

MATERIAL AND METHODS

Tissues

Twenty seven trophoblast samples were obtained from curettages performed at the Department of Obstetrics and Gynecology, University of Liège, CHR Citadelle Hospital, Liège, Belgium. Trophoblast tissue samples were briefly rinsed in 0.9 % saline solution, the terminal villi were dissected from the trophoblastic bulk, and snap frozen in liquid nitrogen. The samples were classified by gestational age, according to ultrasonographic measurements. Pathologic examination confirmed the normal morphology of the samples. At least three samples were studied in each class of gestational age.

RNA extraction and Northern blot analysis

Total cellular RNA was isolated from the tissue powder of trophoblast samples using the guanidine isothiocyanate extraction procedure and cesium chloride gradient centrifugation as described (9). Equal amounts (5µg) of total RNA were separated by size through a 1.2% (wt/vol) agarose-formaldehyde gel and transferred to nitrocellulose filters as described (6). 67LR and Galectin-3 cDNA probes have already been described (7, 10). cDNA inserts were purified, [^{32}P] labeled by nick translation and hybridized to the filters as described (6). Hybridization signals on the blots were quantified by the Scan Analysis program for the Macintosh (BioSoft, Cambridge, UK). The mRNA level for each gene product was normalized to densitometric readings of the GAPDH mRNA levels and were expressed in relative optical density units.

Immunoblots

Total trophoblast protein extracts were prepared in 1% SDS using a Dounce homogenizer. Equal amounts of protein, as determined using a bicinchoninic acid protein assay (Pierce Chemical Co, Rockford, IL), were dissolved in reducing sample buffer, separated by electrophoresis on 10% SDS-polyacrylamide gels, and transferred to a polyvinylidene difluoride membrane (Immobilon, Millipore Corp., Bedford, Mass.) as described (11). The 67LR was detected using the previously described affinity-purified antibody 4099 (11). Galectin-3 was detected with an anti-galectin-3 antibody (12). The immunoblots were performed using an enhanced chemiluminescence (ECL) Western blotting detection system (Amersham). Quantification of the level of expression of each protein was performed by densitometric analysis of the immunoblots as described above for the assessment of RNA levels.

RESULTS

67LR expression correlates with trophoblastic invasion

The expression of the 67LR in trophoblastic samples was examined using Northern and Western blotting. As expected, the 67LR probe detected a single 1400 bases mRNA on Northern blots (Figure 1A). Densitometric analysis of these blots demonstrates that 67LR expression increased progressively in trophoblastic samples from the 7th week of pregnancy, reaching a peak at the 12th week, and then decreased at the 13th week of gestation (Figure 2A). The expression of the 67LR at 12 weeks was approximately equivalent to 250 % of the expression at 7 weeks. This pattern of expression correlates directly with the invasiveness of the trophoblastic tissue.

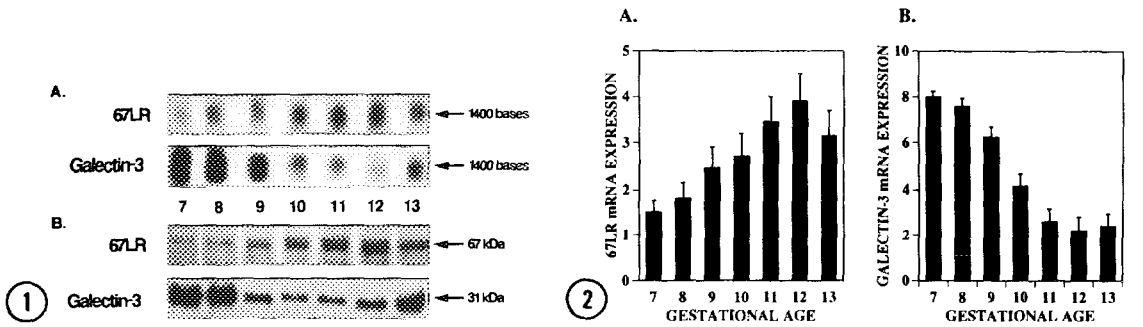

Figure 1. Inverse modulation of 67LR and galectin-3 expression in representative trophoblast tissue samples according to the gestational age: the 67LR expression reaches a maximal value, and the galectin-3 a minimal value, at 12 weeks of gestation. RNA were extracted and analyzed by Northern blotting with 67LR and Galectin-3 cDNA probes as described in "Material and Methods" (Panel A). Protein extracts were analyzed by Western blotting using antibody 4099 and anti-galectin-3 antibody (Panel B). The gestational age is expressed in weeks.

Figure 2. Expression of the 67LR (A) and galectin-3 (B) in trophoblast tissue as defined by Northern Blotting. The values correspond to the mean and standard deviations of the ratios between the 67LR (A) or galectin-3 (B) mRNA levels and the values corresponding to the housekeeping gene GAPDH in the trophoblast samples. 67LR expression increases to reach a peak at 12 weeks, whereas galectin-3 expression decreases to a minimal value at 12 weeks. The gestational age is expressed in weeks.

Western blots performed with the 4099 antibody revealed that the protein levels followed the same pattern that mRNA levels, i.e. increased expression of the 67LR such as in invasive tissue (Figure 1B).

Galectin-3 expression is inversely correlated with trophoblastic invasion

As for the 67LR, Western and Northern blots were performed to evaluate the expression of galectin-3 in the trophoblastic samples (Figure 1). Evaluation of galectin-3 mRNA levels detected by the cDNA probe on Northern blots revealed that its expression was inversely modulated as compared to the 67LR. Indeed, the level of expression of galectin-3 decreased from the 7th gestational week to a minimal expression present at the 12th week, followed by a slight increase at the 13th week (Figure 2B). The expression of galectin-3 at 12 weeks was decreased by 75 %, compared to the level of expression at 7 weeks.

The anti-galectin-3 antibody detected a single 31 kD band on immunoblots (Figure 1B). Expression of galectin-3 protein in trophoblast samples as detected by this antibody on Western blots followed the same trend than the corresponding mRNA levels (Figure 1B).

DISCUSSION

Trophoblastic cells invade the normal tissues, like cancer cells, but in a spatially and temporally limited fashion (1). Several cancer cell invasion related gene products have been already studied in trophoblastic invasion, such as integrins (13) and type IV collagenases (3, 4). To date, no information about the expression of two non-integrin laminin binding proteins, the 67LR and galectin-3, was available. The expression of these two proteins has been already examined in a variety of human cancers, including colon (6, 7), ovary (14) and breast carcinoma (manuscript in preparation). It was shown that the 67LR was upregulated, whereas galectin-3 was downregulated in the cancer lesions compared to the corresponding normal tissues (6, 7). This inverse modulation correlates with the invasive and metastatic phenotype of the cancer lesions.

Invasion is a complex process that involves the coordinated up and down expression of a large variety of genes. It is likely that the invasive phenotype results from the application of a genetic program that orchestrates the regulation of these genes. We postulate that cancer cells use in a non controlled fashion this invasive phenotype program. This has been supported by previous studies showing that, as in cancer, genes such as metalloproteinases (3, 4) and integrins (13) are upregulated in trophoblast.

Our present study demonstrates that the pattern of expression of the 67LR and galectin-3 progresses to the pattern observed in cancer cells as trophoblast cells exhibit their most invasive characteristics. These data add weight to the body of evidence that upregulation of the 67LR and coordinated downregulation of galectin-3 result from the activation of the genetic program that leads to the acquisition of invasive properties by cells. Our study confirms the trophoblast as an appropriate model to elucidate the molecular mechanisms that sustain the invasive process. The identification of the pathways that coordinate the up and down regulation of the 67LR and of galectin-3 could potentially lead to the identification of the master genes coordinating the invasion process and, hence, to the development of new anti-invasive therapeutic strategies.

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