Galectin-3 Expression in Human Endometrium and Decidua During The Menstrual Cycle and in Gestation

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OBJECTIVE: G3 is one of the members of the lectin family that may be involved in the establishment of endometrial receptivity by regulating the proliferation and adhesion of endometrial cells.

STUDY DESIGN: Immunohistochemical expression of G3 was tested in late secretory phase of the endometrium (SPE)(n=30), proliferative phase of the endometrium (PPE)(n=34), and decidua (n=41) and induced abortions in the first trimester.

RESULTS: Expression of G3 during the PPE was low; 12 cases were score 1 and 19 cases were score 2 ($p<0.001$). However, among the late SPE, there were 11 cases at score 2, and 15 cases at score 3. In the decidua expression of G3, 37 cases were score 3 ($p<0.001$).

CONCLUSION: There is a significant progression of G3 expression in SPE and gestation. Although during the PPE expression is considerably lower. This shows that G3 plays an important role in implantation.

Key words: Endometrium, Galectin-3, Secretory, Proliferative, Decidua.

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Introduction

The endometrial cycle and implantation are complex processes. A successful establishment of endometrial receptivity requires endometrial development, proliferation and differentiation.[1] Endometrial cells first undergo proliferation and then become differentiated, increasing their adhesive ability. Alterations in the expression of some regulatory molecules on the cell surface occur during the cycle tightly controlled proliferation/differentiation. Most of these regulatory molecules and mechanisms have not yet been investigated, however.

Protein (lectin)–carbohydrate interactions are delineated to regulate crucial cell functions. Galectins are members of a large family of endogenous lectins, which contain one or more carbohydrate recognition domains with an affinity for β-galactosides.[2] Galectin-3 is effective in cell growth, differentiation, apoptosis, the cell-cell and cell-extracellular matrix relationship, chemoattraction, and migration.[3] It is important in normal cell growth and tumorigenesis. Galectin-3 has been shown to induce differentiation and angiogenesis of endothelial cells.[4] Galectin-3 is also a chemoattractant for monocytes [5] and endothelial cells.[4] It is expressed at the maternofetal interface[6] and in placental trophoblasts.[7,8] Galectin-3 protein is found in uterine epithelial cells adjacent to implanting blastocysts in different cell types. Several reports have shown that galectins are important mediators of inflammation.[9] As endometrial function and implantation involve many inflammatory mediators, galectins might contribute to the modulation of the endometrial immune system. In addition, galectin-3 has been shown to play an important role in cell adhesion, migration, and chemotaxis.[9] As these functions are essential for the regulation of the endometrium, we set out to analyze the currently known human galectin-3 expression throughout the menstrual cycle and early gestational decidua.

Material and Method

Cases

Specimens of endometrial tissue (n=64) from women with

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regular menstrual cycles were collected at different phases of the cycle. Of these, 34 cases had proliferative phase endometrium and 30 cases had late secretory phase endometrium. Curettage was performed for benign reasons. Decidual tissue (n=41) was obtained from induced abortions in early gestation. In total, 105 cases from the Istanbul Education and Research Hospital, and Kartal Dr. Lütfi Kırdar Education and Research Hospital were used in our study. Tissues were fixed in formaldehyde and blocked with paraffin.

Galectin-3 Immunostaining

A mouse monoclonal antibody to galectin-3 (31 kD β-galactoside binding lectin, monoclonal; Novocastra Laboratories, Newcastle, UK) against mouse myeloma protein was applied. Antiserum to galectin-3 was used at 1:1000 dilution. Representative sections of 4μm obtained from paraffin blocks were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 3% H_2O_2 diluted in methanol. Sections were incubated overnight in a humid chamber with mouse monoclonal antibody anti-galectin-3, clone 9C4 (Novocastra Laboratories, UK) at 4°C. Sections were then incubated with biotinylated secondary antibody (DAKO, USA) for 30 minutes at room temperature. Indirect immunoperoxidase was carried out using a commercially available avidin-biotin staining kit (DAKO, USA). The immunoenzymatic activity was developed with 3,3'-diaminobenzidine (DAB) tetrahydrochloride solution. Hematoxylin was used for counterstaining.

Immunohistochemical Scoring

A semiquantitative scale was used:

0- No staining

1- Focal staining or positive staining seen in some of the cells

2- Heavy staining in at least one focus or intermediate to light staining in many foci

3- Heavy staining in more than half of the cells (Figure 1-3).[7]

Statistical Analysis

All statistical tests were performed using the SPSS ver. 10.0 data analysis program (SPSS Inc, Chicago, IL, US). Data were analyzed using the Kruskal–Wallis test.

Results

The mean age of the patients was 37.6 and the median age was 39. Statistically, the mean and the median ages of score 3 galectin-3 cases were significantly lower than score 1+ and 2+ cases ($p<0.001$).

The mean age of the cases showed that score 3 staining with galectin-3 was 33.26; the median was 34.50. Among the cases with score 1 staining, the median age was 42.50 and the mean was 41.50.

Twelve samples of proliferative endometrial tissue showed score 1 staining (Figures 1), 19 showed score 2 staining (Figures 2) and only 3 showed score 3 staining. Expression of galectin-3 during the proliferative phase was considerably lower ($p<0.001$) .

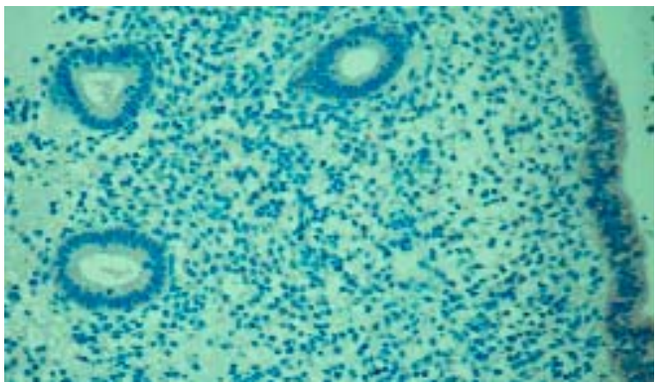


Figure 1: Focal immunoreactivity of galectin-3 in some of the cells in proliferative endometrium (Score 1 staining).

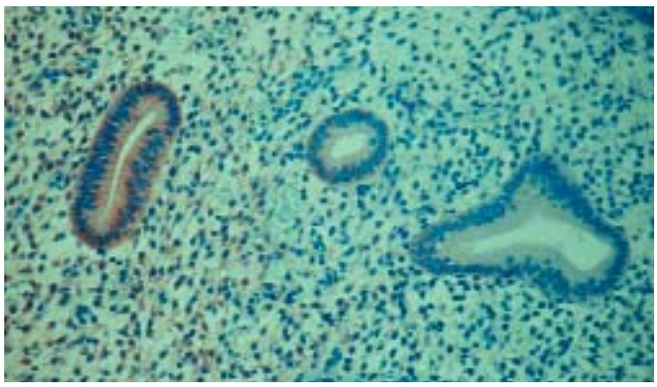
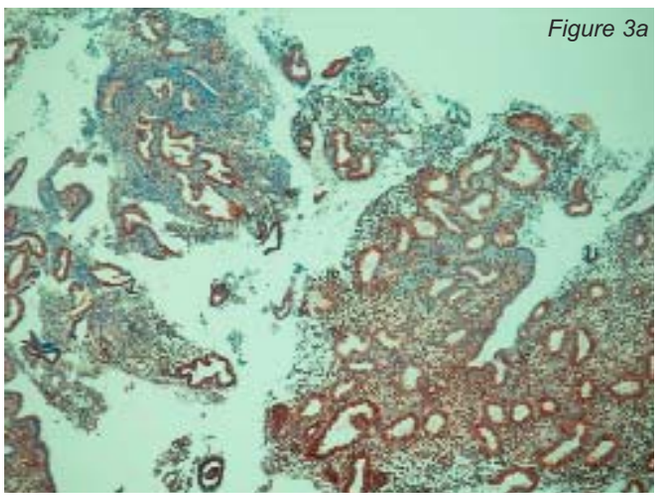


Figure 2: Intermediate to light staining in many foci in proliferative endometrium (Score 2 staining)

Four of the late secretory tissue cases showed score 1 staining, 11 showed score 2, and 15 showed score 3 (Figures 3a). The decidual tissues showed only score 2 and score 3 staining. Of the tissue cases, 4 were score 2 and 37 were score 3 (Figure 3b).

Figure 3a



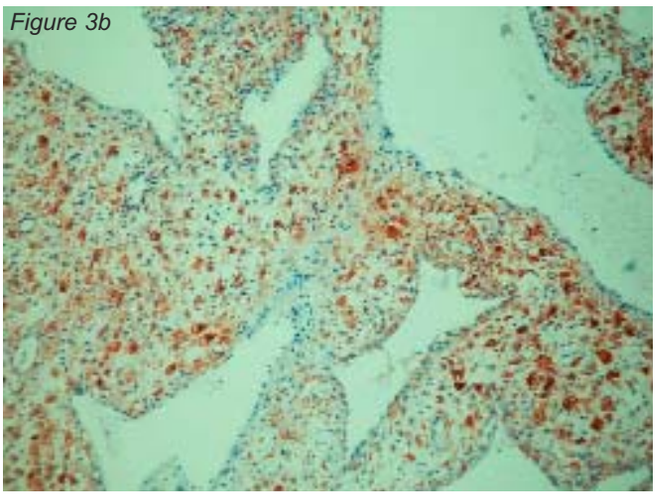


Figure 3: Heavy staining in more than half of the cells (Score 3 staining). 3a; Score 3 immunostaining in secretory endometrium. 3b; Score 3 immunostaining in decidua.

Expression of galectin-3 increased significantly in the late secretory phase endometrium and in the decidual tissue ($p<0.001$) (Table 1).

Discussion

This study provided comprehensive information on galectin-3 in cyclic endometrium and adhesion. In the pre-implantation period, the uterine tissue must be suitable for embryonic implantation. Alterations during the endometrial cycle and early gestation are primarily controlled by estradiol and progesterone. In addition, growth factors and cytokines are involved in endometrial regulation by autocrine and paracrine mechanisms.

Galectin-3, one of the members of the β-galactoside binding lectin family, binds to basal membrane glycoprotein and plays a role in many biological events.[3] Galectin-3 has been shown to activate various cell types through cross-linkage of appropriate cell surface glycoproteins, and to modulate cell adhesion, probably through interaction with cell surface glycoproteins.[10] Galectin-3 is an important mediator of inflammation.[3] It has been shown to induce differentiation and angiogenesis of endothelial cells.[4] Galectin-3 is also a chemoattractant for monocytes[5] and endothelial cells.[4]

Previous studies have suggested that galectin-3 is expressed in uterine epithelial cells adjacent to implanting blastocysts, as well as in the decidualized endometrium of implantation sites.[11-13] Targeting such an extracellular matrix of glycoproteins and membrane glycoproteins, which are produced by human blastocysts and trophoblasts,[14,15] can be a function of galectin-3. Fittingly, the detection of galectin-3 as a stage-specific gene product in bovine pre-implantation development [16] raises respective evidence. With the availability of galectin-3 as a tool to monitor the profile of accessible binding sites, future work could map the presence of cellular targets for the tissue lectin. In addition to glycans, intracellular proteins with specificity to a galectin will also be detected.[17, 18] In this study, we detected a high level of galectin-3 in the decidual endometrium.

Expression of galectin-3 increased significantly during the late secretory phase of the menstrual cycle and in the decidua of early gestation.[19] Galectin-3 modulates cell adhesion by binding to several ligands, including laminin, fibronectin, and integrins, after its secretion from epithelial cells.[20] Furthermore, galectin-3 is expressed at the maternofetal interface[6] and in placental trophoblasts.[8] The invading, galectin-expressing trophoblast cells interact with galectin-expressing epithelial and decidualizing stromal cells. Lei et al. showed that a deficit in endogen galectin-3 reduced endometrial cell proliferation and adhesion.[21] In addition, in our study, expression of galectin-3 was significantly higher in the endometrial stroma during the secretory phase of the menstrual cycle and in the decidua of gestation. The increase of galectin-3 expression in the decidua of early gestation was significantly higher than in the late secretory phase of the menstrual cycle.

In one investigation, it was suggested that galectin-3 is absent in non-decidualized endometrium[12] and only present in pregnancy. But in our study, all the tissues, in both pregnancy and non-gestational endometrium, showed galectin-3 expression. However, the density of galectin-3 expression was higher in the decidualized tissue than in the normal cyclic endometrium. We suggest that galectin-3 is present in both the normal and gestational endometrium, but the higher density in the late secretory phase and decidualized endometrium prove that galectin-3 is a supportive factor at the time of implantation of the embryo.

Table 1: Expression of galectin-3 in proliferative, secretory endometrium and decidua.

	- Galectin-3 expression	1+ Galectin-3 expression	2+ Galectin-3 expression	3+ Galectin-3 expression	Total
Secretory endometrium	0	4	11	15	30
Proliferative endometrium	0	12	19	3	34
Decidua	0	0	4	37	41
Total	0	16	34	55	105 ($p<0.001$)

There was no demographic data regarding the density of galectin-3 in the endometrium. In our study, we found that the expression of galectin-3 is higher at younger ages and decreases with age.

In conclusion, the expression of galectin-3 is considerably lower during the proliferative phase and at older ages and is significantly higher in the late secretory phase endometrium and decidual tissue and at younger ages. Taken together with previous studies, our study suggests that this lectin plays an important role in implantation and gestation.

İnsan Endometriumunda Mensturual Siklusta ve Gestasyonda Galektin-3 Ekspresyonu

AMAÇ: Lektin ailesinin üylerinden biri olan Galektin-3 (G3) endometrial hücrelerin adezyonunu ve proliferasyonunu düzenleyerek endometrial resepsivitenin oluşumuna dahil edilebilir.

GEREÇ VE YÖNTEM: G3'ün endometriumun geç sekretuar (SPE) (n=30), proliferative (PPE) (n=34) fazında, ve ilk trimester abortus materyallerinin desidual dokularında (n=41) immunhistokimyasal olarak G3 ekspresyonu test edildi.

BULGULAR: G3'ün PPE'deki ekspresyonu düşüktü, 12 olgu skor 1, 19 olgu skor 2'idi (p<0,001). Ancak geç SPE olgularında 11 olgu skor 2, ve 15 olgu skor 3 izlendi. Desiduada ise 37 olgu skor 3 boyanma gösterdi (p<0,001).

SONUÇ: SPE ve gestasyonda G3 ekspresyonunda belirgin progresif bir ekspresyon mevcuttur. Ancak PPE SIRASINDA ekspresyonbelirgin olarak düşüktür.. Bu G3'ün implantasyonda önemli bir rolü olduğunu göstermektedir.

Anahtar Kelimeler: Endometrium, Galectin-3, Proliferative, Sekretuar, Desidua.

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