Original Article

Comparative Immunohistochemical Study of Galectins 1, 3 and 9 in Ovarian Epithelial Neoplasms

*Ana Cristina da Rocha Duque[1], Élia Cláudia de Souza Almeida[1], Sanívia Aparecida Lima Pereira[1], Eddie Fernando Candido Murta[2], Rosekeila Simões Nomelini[2], Cléber Sérgio da Silva[2] and Renata Margarida Etchebehere[3]**

[1]*Post Graduate Program in Health Sciences, Triângulo Mineiro Federal University (UFTM), Uberaba, Minas Gerais, Brazil*
[2]*Discipline of Gynecology and Obstetrics, Triângulo Mineiro Federal University (UFTM), Uberaba, Minas Gerais, Brazil*
[3]*Surgical Pathology Service, Clinical Hospital, Triângulo Mineiro Federal University (UFTM), Uberaba, Minas Gerais, Brazil*

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ABSTRACT

Ovarian neoplasms are among the most common in the female genital tract and often have delayed diagnosis. Tumor progression involves signalling proteins called galectins. The aim of the present study was to evaluate the immunohistochemical expression of galectins "1", "3" and "9" in the ovarian surface epithelial neoplasia. A retrospective study involving 62 ovarian epithelial tumors (benign and non-benign) was performed with immunohistochemical polymer technique and antibodies against galectin "1", "3" and "9". Expression in epithelium and stroma was analysed semi-quantitatively. Fisher's exact test was performed for statistical analysis. Galectin-"1" and "3" were strongly expressed in non-benign tumors of the epithelium. Non-benign neoplasms showed increased stromal expression of galectin-1 and increased epithelial expression of galectin-"3". The significant increase in expression of galectin-1 and -3 in the epithelium of non- benign ovarian neoplasms suggests the participation of these galectins in ovarian carcinogenesis. We observed increased stromal expression of galectin-"1" and epithelial expression of galectin-"3" in non-benign ovarian neoplasms. These findings contribute to knowledge about the role of these galectins in the growth and spread of ovarian cancer.

Introduction

Ovarian neoplasms occur with high incidence in the female genital tract [1]. About 75% are initially diagnosed at an advanced stage. Ovarian neoplasms account for about 5% of cancer deaths among women (more than any other gynecological cancer). Because of the high incidence of this neoplasm, diagnosis is invariably delayed, leading to high morbidity and high lethality. Identification of markers associated with ovarian cancer is necessary to improve treatment for this disease [1, 2].

Galectins (Gal) are one type of lectin. Lectins are signaling proteins [3-5] that participate in several physiological and/or pathological functions such as cell cycle progression, apoptosis, innate and adaptive immunity, and messenger RNA processing [6-10]. Gal has been studied in association with several types of cancer, with Gal-"1" and "3" being the most commonly studied. Some neoplasms accumulate large amounts of Gal-"3". On the other hand, Gal-"3" is not universally associated with tumor progression; expression levels are diminished in breast, colon and skin tumors [6-10]. It is speculated that Gal-"3" expression plays some role in late-stage tumor progression, favouring chemoresistance and development of metastases [11]. Similarly, increased Gal-"1" expression is common in different types of cancer, particularly in metastatic tumors [5, 12]. Gal-"9" is reported to be involved in carcinogenesis in other different tumor [13]. It is not yet established in the literature whether Gal plays an important role in ovarian carcinogenesis. In addition, we did not find studies on the expression of Gal-"9" in these tumors. This justifies the present study, which intends to evaluate immunohistochemical expression of Gal-"1", "3" and "9" in ovarian surface epithelial neoplasms.

Correspondence to: Renata Margarida Etchebehere, Surgical Pathology Service, Clinical Hospital, Triângulo Mineiro Federal University (UFTM), Rua Getúlio Guaritá 130, Uberaba, Minas Gerais, ZIP Code 38025440, Brazil; Tel: 553433185152; E-mail: renataetch@hotmail.com

Materials and Methods

Sixty-two cases of ovarian epithelial neoplasia treated at a public tertiary hospital were analysed: 39 serous and mucinous benign epithelial neoplasms and 23 non-benign serous and mucinous epithelial neoplasms (9 cystadenocarcinomas and 14 borderline tumors). Nine patients were staged as grade III; 14 were staged as grade I or II (International Federation of Gynecology and Obstetrics). No patient included in the study had undergone chemotherapy prior to diagnosis. Cases were selected based on reports issued by medical pathologists at the time of surgery. Original slides were reviewed, and no instance of divergence from initial diagnosis was noted. In cases of tumors in both ovaries, the sample from only one side was used. When sides differed in terms of diagnosis, the sample most representative of the neoplasia and/or more aggressive histological behaviour was selected.

Sample size was determined by accessibility, especially for borderline and malignant neoplasms. Participants age ranged from 17 to 79 years (median 40.5) in patients with benign neoplasms and 33 to 83 years (median 52.7) in the non-benign group. Gal-"1", "3" and "9" expression in neoplastic tissues was identified through immunohistochemical polymer technique (Spring Bioscience, CA) with anti-Gal-"1" (R&D Systems, Minneapolis, MN) dilution 1:6000, anti-Gal-"3" (R&D Systems, Minneapolis, MN) at 1:200, and anti-Gal-"9" (DAKO, Carpinteria, CA) at 1:350 dilution. It was not possible to evaluate Gal-"1" expression in one case of non-benign neoplasia and Gal-"9" expression in one case of benign neoplasia because of the scarcity of material. For quantification of labelled cells, we used the scoring criteria adopted by Etchebehere *et al*. (2018) [14]. Slides were evaluated by two independent observers and categorized according to the ratio of stained cells: 0=absent; 1=rare; 2=moderate; 3=abundant. For quantification of epithelial cells, cytoplasm and nucleus were considered. For quantification of stromal cells, underlying epithelial stromal cells were included for benign neoplasms; peritumoral stromal cells were included for non-benign cases.

We used the software BioStat ® (version 5.0) for statistical analysis. Two-by-two comparisons among groups were performed with Fisher's exact test. Results were considered significant when the probability of rejection of the null hypothesis <5% ($p<0.05$). Kappa coefficient was used to calculate agreement between observers. For statistical purposes, scores 0 and 1 were considered "weak" and scores 2 and 3 were considered "strong".
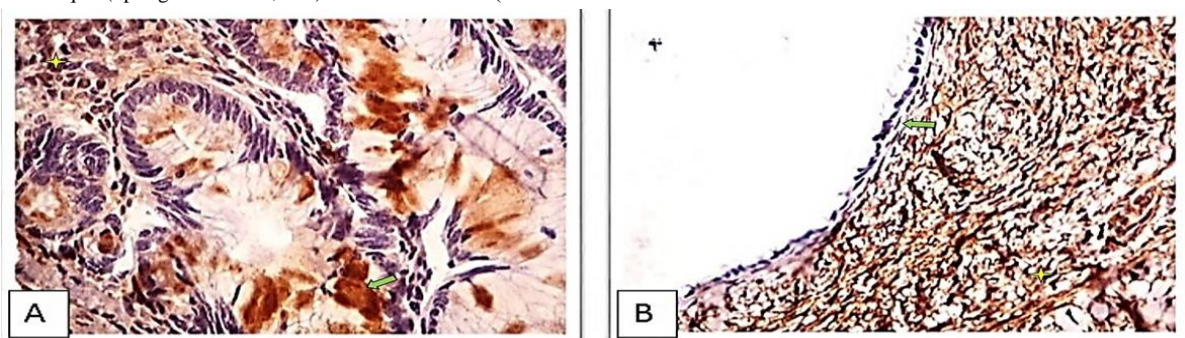

Figure 1: A) Histological section of non-benign epithelial ovarian tumor showing strong expression of Gal-1 in epithelium (arrow) and stroma (star) of neoplasia (polymer technique, 400X); **B)** Histological section of non-benign epithelial ovarian tumor showing weak expression of Gal-1 in epithelium (arrow) and strong expression in neoplastic stroma (star) (polymer technique, 100X).

Figure 2: A) Histological section of non-benign epithelial ovarian tumor showing strong expression of Gal-3 in epithelium (arrow) and weak expression in neoplastic stroma (star) (polymer technique, 400X), **B)** Histological section of non-benign epithelial ovarian tumor showing weak expression of Gal-3 in epithelium (arrow) and strong expression in neoplastic stroma (star) (polymer technique, 100X).
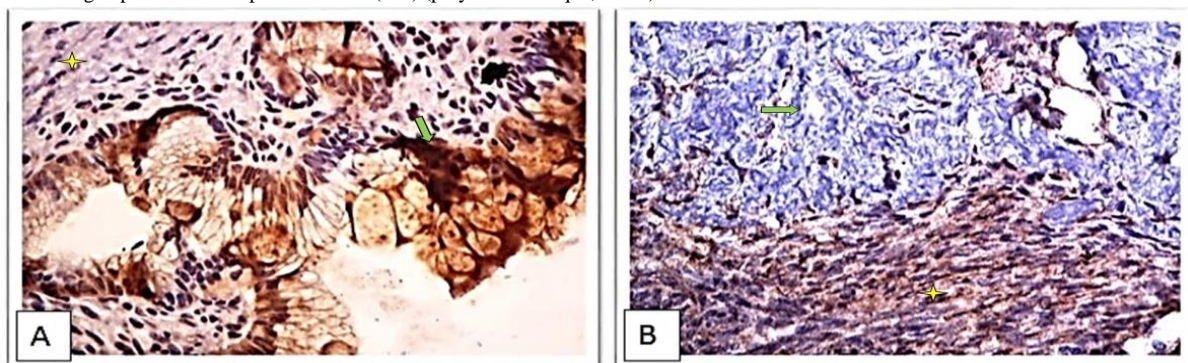
Results

The Kappa coefficient for the three markers was 0.9. Divergent cases were decided upon through joint evaluation by the observers. Table 1 shows the immunohistochemical expression of the antibodies studied with respect to their intensity (weak or strong) in epithelial and stromal cells of the benign and non-benign neoplasms evaluated. A significant strong epithelial expression of Gal-"1" and "3" (Figures 1 & 2) respectively, was observed in non-benign neoplasms when compared to

benign. Expression of Gal was similar to that observed in stromal cells. No difference in Gal-"9" expression (in epithelium or stroma) was observed between groups.

Table 1: Immunohistochemical expression of Gal-"1", "3" and "9" in epithelium and stroma of benign and non-benign ovarian neoplasms.

Galectins	Histological behavior			
	Expression	Benign	Non-benign	
Gal – 1 Epithelial	Weak	32	7	*
	Strong	7	15	
Gal – 3 Epithelial	Weak	30	8	**
	Strong	9	15	
Gal – 9 Epithelial	Weak	34	18	
	Strong	4	5	
Gal – 1 Stromal	Weak	7	1	
	Strong	32	21	
Gal – 3 Stromal	Weak	34	21	
	Strong	5	2	
Gal – 9 Stromal	Weak	36	22	
	Strong	2	1	

* p=0,00; ** p=0,00; Fisher's exact test.

Table 2: Intensity of immunohistochemical expression of Gal-"1" and "3"in epithelium and stromal cells of non-benign ovarian neoplasms .

	Non-benign Neoplasias		
	Expression	Epithelial cells	Stromal cells
Gal-1	Weak	7	1	*
	Strong	15	21	
Gal-3	Weak	8	21	**
	Strong	15	2	

* p=0,0459; ** p=0,0001; Fisher's Exact Test.

Table 2 shows immunohistochemical expression of Gal-"1" and "3" in relation to staining intensity in epithelial and stromal cells of non -benign neoplasms. Comparing the expression of Gal-"1" and "3" in non-benign neoplasms, significant strong expression of Gal-"1" was observed in stromal cells and increased expression of Gal-"3" was observed in epithelial cells.

Discussion

Cancer derives from differentially accumulated mutations in a population of genetically unstable cells that, resulting in uncontrolled cell growth. Studying tumor microenvironment may elucidate the process of malignization. During carcinogenesis and tumor progression, cells acquire properties conducive to establishment and maintenance that differ from those found in normal cells. These properties include maintenance of self-replication, increased cell survival, high genetic instability, dysregulation of energy metabolism, and evasion of the immune system [15].

Although the comprehensive effects of Gal remain to be established, it is clear that these proteins mediate numerous aspects of cell biology. Gal are multifunctional proteins that act in the nucleus, cytoplasm, cell membrane, and extracellular matrix. In recent years, several studies have demonstrated significant expression of Gal in human tumors [16]. There

is scarce data in the literature on Gal-"1" in the context of ovarian cancer. Some authors maintain that expression of Gal-"1" is increased in malignant neoplasms, including malignant ovarian neoplasms, particularly in stroma [17]. Other studies have reported expression of Gal-"1" in 100% of serous, mucinous and endometrioid ovarian tumors samples. Gal-"1" expression was also found in 65% of epithelial and 100% of stromal cells [18]. One study of Gal-"1" expression showed positive staining in 56.6% of tumoral epithelium and 57.1% of stromal tissue [19]. However, the authors do not describe the intensity of immune staining.

In a preliminary study in rats, Nio and Iwangua (2007), observed diffuse Gal-"1" expression in stroma of ovarian tumors. These data corroborate our findings [20]. When we evaluated Gal-"1" expression in non-benign neoplasms, we found significant expression in peritumoral stroma. Gal-"1" expression is common to several types of cancer, including metastatic tumors [5, 12]. We infer that increased stromal expression of Gal-"1" in non-benign tumors relates to behavior of the neoplasia and to the ability of these tumors to invade stroma and generate metastasis. Serum levels of Gal-"1" differed significantly between patients with non-metastatic vs. metastatic epithelial tumors, indicating that an increase in Gal-"1" expression indicates increased aggressiveness of the neoplasia. These findings suggest that Gal could be used as a prognostic marker [21]. In our study, strong expression was maintained in early and advanced non- benign neoplasms, regardless of staging. Other authors also reported higher Gal-"1" expression in ovarian tumors when compared to normal tissue, as well as an association between Gal expression, tumor aggressiveness, and poor prognosis [22].

One study that evaluated expression of Gal-"3" in malignant ovarian epithelial and endometrioid neoplasms reported expression in 88.7% of epithelial cells, with variable expression in adjacent stroma. The authors also reported that Gal-"3" expression varied from moderate to strong in most tumor cells [23]. In the present study, epithelial expression of Gal-"3" was significantly higher in non-benign compared with benign neoplasms. This study reports, for the first time, increased expression of Gal-"3" in epithelium, compared with stroma of non-benign ovarian neoplasms. This trend represents an inverted pattern of Gal-"1" expression. Gal-"3" "may therefore" be related to immortalization of malignant clones, through inactivation of tumor suppressor genes that compromise apoptosis or activation of oncogenes. Increased expression of Gal within the neoplastic cell (epithelium) is likely linked to cell cycle progression. There is evidence that an apoptotic stimulus triggers translocation of Gal-"3" to the mitochondria, which blocks changes in mitochondrial transmembrane potential and thus prevents cell death [24]. Elevated serum levels of Gal-"3" appear to indicate the resumption of tumor growth and cell cycle progression. For example, during chemotherapy, serum levels of Gal-"3" decreased in serous cystadenocarcinoma; after discontinuation of treatment, Gal levels progressively increased [25].

Angiogenesis is another crucial event in the growth and spread of tumors. Nangia-Makker *et al.* (2000) showed *in vitro* participation of Gal-"3" in capillary tubule formation and *in vivo* angiogenesis, suggesting that Gal may act as a pro-angiogenic factor [26]. Based on this evidence, it is impossible to deny the participation of Gal-"3" in ovarian carcinogenesis. Gal-"9" has a role in hemostasis and

inflammation [27]. We did not find studies on expression of Gal-"9" in ovarian neoplasms.

Antitumor activity of Gal-"9" was reported in studies involving neoplasms in other sites [13]. We did not find differences in the expression of Gal-"9" in the epithelium or stroma of benign vs. non-benign tumors, suggesting that Gal-"9" has antitumor activity and does not participate in the progression of ovarian neoplasia.

Conclusion

The strong stromal expression of Gal-"1" and significant increase in expression of Gal-"1" and "3" in epithelium of non-benign ovarian neoplasms suggests participation of Gal-"1" and "3" in ovarian carcinogenesis. Here we have described, for the first time, an inverted pattern of Gal-"3" expression in epithelium and stroma. Additional studies will be necessary to further elucidate Gal's role in tumor growth and ovarian cancer.

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