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From Cell Biology to Tissue Engineering

Aberrant levels of Wnt/β-catenin pathway components in a rat model of endometriosis

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Summary. Endometriosis is a benign gynecological disease affecting approximately 10-15% of women of reproductive age and 25-50% of all infertile women. It is characterized by the presence of glands and/or endometrial stroma outside the uterine cavity. Angiogenesis is a crucial process for the development and maintenance of endometriotic lesions. The Wnt/β-catenin pathway is a major promoter of angiogenesis in both physiological and pathological conditions. In the present study, we evaluated the expression of molecules related to the Wnt/β-catenin pathway in a rat model of peritoneal endometriosis. mRNA analyses showed significantly increased expression of *Wnt4* and *Wnt7b* and decreased expression of *Gsk3beta* and *E-cadherin* in endometriotic lesions. However, there were no differences in *β-catenin* and *Fzd2* mRNA expression. In addition, we observed a significant increase of nuclear β-catenin in endometriotic lesions, a hallmark of Wnt/ β -catenin pathway activation. Stromal β-catenin staining was found in 45.4% of endometrial tissues and 77.8% of endometriotic lesions. β-catenin nuclear localization was found in 18.2% of the endometrial tissues and 33.3% of endometriotic lesions. Finally, the expression of galectin-3, a regulator of this pathway, was increased in endometriosis. In summary, this pattern of Wnt/β-catenin components expression suggests an increased activity of this pathway in endometriosis.

Key words: Endometriosis, Wnt pathway, β-catenin, Galectin-3, Rat models

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Introduction

Endometriosis is a benign sex hormone-dependent gynecological disease that affects 10-15% of women in reproductive age, and 25-50% of infertile women worldwide (Giudice and Kao, 2004). The disease is characterized by the presence and growth of endometriotic lesions, consisting of functional endometrial glands and stroma, outside the uterus. Lesions mostly occur on the pelvic peritoneum, but also on the ovaries and in the rectovaginal septum, and more rarely in the pericardium, pleura, and even the brain (Galle, 1989; Giudice and Kao, 2004). The most common and specific symptom of endometriosis is pain, generally in the form of dysmenorrhea, but also as dyspareunia, dysuria or dyschezia. The second most common symptom is infertility, with monthly fecundity rates reduced as much as tenfold (Asante and Taylor, 2011). The pathophysiology of endometriosis is believed to result from the reimplantation of endometrial fragments retrogradely shed during menstruation (Sampson, 1927), and angiogenesis has been shown as a crucial step for the establishment and growth of endometriotic lesions (Groothuis et al., 2005; Becker and D'Amato, 2007; Taylor et al., 2009).

The Wnt signaling pathway plays an important role in angiogenesis, regulating several processes necessary for the development of new blood vessels, such as cell proliferation and polarity (Zerlin et al., 2008). β-catenin is an important component of the Wnt pathway and can act as a transcription factor for several genes. In the absence of Wnt stimulation, β-catenin is mostly associated with the plasma membrane where, in conjunction with other molecules, it promotes cell adhesion. When β-catenin is freed from the adhesion complex, it is phosphorylated by glycogen synthase kinase 3β (GSK3β)/Axin/adenomatosis polyposis coli (APC) and subsequently degraded by the proteasome. However, when Wnt family members bind to frizzled (FZD) receptors and low-density lipoprotein receptor–related protein (LRP) 5 or LRP6 co-receptors, β-catenin phosphorylation and degradation are inhibited. Therefore, β-catenin accumulates in the cytoplasm and translocates to the nucleus, where it interacts with transcription factors to activate transcription of target genes (Kimelman and Xu, 2006; Takemaru et al., 2008; MacDonald et al., 2009), including pro-angiogenic molecules such as vascular endothelial growth factor (VEGF) and interleukin-8 (Zhang et al., 2001; Easwaran et al., 2003; Li et al., 2003).

Despite the evidence that Wnt/β-catenin signaling induces angiogenesis, and the role of angiogenesis in endometriotic lesions, only a few inconclusive studies have attempted to associate alterations of this pathway with endometriosis. In the present study, we evaluated the expression of molecules related to the Wnt/β-catenin pathway in an experimental model of peritoneal endometriosis. Our data show an outstanding presence of β-catenin in the nucleus in endometriotic lesions, suggesting an activation of the canonical Wnt/β-catenin signaling pathway.

Materials and methods

Animals

Animals were treated in accordance with protocols approved by the Institutional Animal Care of the Federal University of Rio de Janeiro (IBCCF-079/14) and the Australian code for the care and use of animals for scientific purposes 8th edition (2013). Female Sprague-Dawley rats (200-250g) with free access to water and food were included in this study, after reaching maturity at 8 weeks of age.

Surgical induction of endometriosis

Twenty female rats were used in the experimental induction of endometriosis, using an adapted method from Vernon and Wilson (1985). For the induction of endometriosis, we used cycling animals with intact ovaries and only selected animals in the estrus stage. For this purpose, the cycle stage was evaluated by vaginal lavage and cytologically examined. Animals were anesthetized with intraperitoneal injection of Dopalen (ketamine) and Anasedan (xylazine) (Paulínia, SP). The abdomen was opened through a 3-cm midline incision to expose the uterus. One uterine horn was ligated at both the uterotubal junction and the cervical end, and was removed. The segment was placed in phosphate-buffered saline at 37°C and split longitudinally, and 5×5-mm pieces were sectioned. These explants were then anchored onto the peritoneum on the right side of the ventral abdominal wall by nonadsorbable polypropylene sutures (Fig. 1A) (Prolene 6-0; Ethicon, Piscataway, NJ). The abdomen was closed and the animals were allowed to recover from anesthesia. The animals were euthanized 30 days after the surgery to determine the attachment and viability of endometrial explants, and to collect these lesions (Fig. 1B). Also, from each experimental animal, tissue samples of eutopic endometrium were obtained for establishing the control group. After dissection, samples from 10 rats were embedded in OCT media (Torrance, CA) and frozen for histological and immunohistochemical studies and the other 10 samples were frozen in liquid nitrogen for qPCR and Western Blot analyzes.

Histology and immunofluorescence

Ectopic and eutopic tissues previously embedded in OCT media were cut (3-micrometers-thick) and placed on silane-treated slides. Then, the sections were fixed for 20 min in acetone (Vetec, Rio de Janeiro, RJ) at -20°C. Part of the sections were stained with Harris' hematoxylin and eosin (Próquimios, Rio de Janeiro, RJ), and examined microscopically for the presence of histological hallmarks of endometriosis, such as endometrial glands and stroma (Fig. 1C-F). The other OCT-embedded tissues were used for immuno-fluorescence staining. Antibody nonspecific binding was blocked with PBS/BSA 5 % and then slices were incubated with an anti-β-catenin primary antibody (1:100, C2206 Sigma-Aldrich, St. Louis, MO) overnight. After that, slices were washed with PBS and incubated for two additional hours with goat anti-rabbit Alexa 546 (1:500, Invitrogen, Waltham, MA). Cell nuclei were counterstained with DAPI (1:20000, Santa Cruz Biotechnology, Dallas, TX). Finally, cells were washed in distilled water and mounted on histological slides with N-propylgallate (Sigma-Aldrich, St. Louis, MO). In all immunostaining-negative controls, reactions were performed by omitting the primary antibody. No reactivity was observed when the primary antibody was absent. Images were captured using confocal microscopy (Olympus IX81) and a Hamamatsu orcaR2 digital camera. Finally, we analyzed the endometria and the endometriotic lesions from 10 animals. For nuclear beta-catenin localization analysis the examiners used all images captured in the confocal microscope, in order to exclude the possibility of false positives by overlapping images. The images were analyzed in the program "Leica application suite (Las AF)" and the statistical

analyses were performed using the GraphPad Prism 5.0.

Real-time PCR

m-RNA levels were quantified by real-time reverse transcription-polymerase chain reaction, as follows. RNA from endometriosis and endometrium samples from the same animal was isolated using the Trizol® reagent (Invitrogen, Waltham, MA) according to the manufacturer's instructions, and quantified by the Nanodrop® spectrophotometer. One microgram of total RNA was subjected to reverse transcription with a commercially available kit (the cDNA First Chain Amplification System, GIBCO-BRL) according to the manufacturer's protocol; approximately 200 ng of cDNA were used for each amplification of oligonucleotides specific. The expression of *β-catenin, E-cadherin, Wnt4, Wnt7b, Fzd2, Gsk3beta* and *Galectin-3 genes* was analyzed using SYBR Green system (SYBR™ Green PCR Master Mix, Applied Biosystems, Waltham, MA) and primers (Table 1). Each sample was analyzed in triplicate. Relative mRNA levels were calculated using the comparative threshold cycle (CT) with the analyzed gene expression levels normalized by those of GAPDH.

Protein extraction and Western blots

Total protein was extracted from ectopic and eutopic tissues using RIPA buffer and protein concentration was quantified using the BCA protein assay kit (Thermo Scientific, Waltham, MA). Cytoplasmic and Nuclear extracts were isolated from ectopic and eutopic tissues by adding first buffer A containing Tris pH 7.4 (20 mM), KCl (10 mM), EDTA (1 mM), glicerol (10%), NP-40 (0.2%), β-mercaptoethanol (0.6 mM) and protease inhibitors (Protease Inhibitor Cocktail; Thermo Scientific, Waltham, MA) to tissues samples which were homogenized using a tissue grinder. Samples were kept on ice for 10 min and then centrifuged at 15600 g for 5 min at 4°C. Cell membrane disruption released cytoplasmic contents in the supernatant which was collected. Next, to the cell pellet left was added buffer B containing Tris pH 7.4 (20 mM), KCl (10 mM), EDTA (1 mM), glicerol (20%), NaCl (0.4 M), β-mercaptoethanol (0.6 mM) and protease inhibitors (Protease Inhibitor Cocktail; Thermo Scientific, Waltham, MA). Samples were kept on ice for 45 min and then centrifuged at 15600g for 10 min at 4°C and supernantant representing the nuclear protein extract was collected. Cytoplasmic, nuclear and whole-tissue protein extracts were resolved by SDS-PAGE and transferred to a polyvinylidene membrane (Bio-Rad, Hercules, CA) using an electroblotter (Bio-Rad). Membranes were blocked with 5.0% nonfat milk for 1 h at room temperature, followed by overnight incubation at 4°C with primary antibodies to β-catenin (C2206, 1:2500, Sigma-Aldrich, St. Louis, MO), GSK3β (#9315, 1:2000, Cell signaling, Danvers, MA) and Galectin-3 (clone M3/38, 1:2000; American Type Culture Collection, Manassas, VA). Primary antibody binding was detected using a HRP-conjugated secondary antibody (anti-rabbit, 1:2000, Southern-Biotech, San Diego, CA; anti-mouse, 1:3000, Amersham Biosciences, Buckinghamshire, United Kingdom) and Immunobilon Western Chemiluminescent HRP substrate (Millipore, Billerica, MA). Internal control was performed by incubating the membranes with β-actin antibody (Sigma-Aldrich, 1:2000, St. Louis, MO), which was also used to confirm cytoplasmic protein extract separation. Anti-laminA/C antibody was used as a control of nuclear protein extract separation (Cell Signaling, #4777, 1:1000, Danvers, MA). The incubation of the antibodies anti-β-actin and anti- laminA/C was followed by incubation with peroxidase-conjugated antibody, and the reaction was developed as described above. Densitometric analysis of bands was performed using the software Image J (http://rsb.info.nih.gov/ij/).

Data analysis

The statistical analyses were performed using the GraphPad Prism 5.0 (GraphPad Software Inc., USA). In the gene expression analysis as well as in western blot assays the data were analyzed using the Student t test. Values were considered statistically significant when $p<0.05$.

Table 1. Sequences of the oligonucleotides used for GSK-3β, β-catenin, E-cadherin, Wnt4, Wnt7b, Fzd2, Galectin-3 and GAPDH amplification by Real-time quantitative RT- PCR (qRT-PCR).

	Forward	Reverse
GSK-3β	5'-TTGGAAATGGGTCATTTGGT-3'	5'-TCACAGGGAGTGTCTGCTTG3'
β-catenin	5'-GCCAGTGGATTCCGTACTGT-3'	5'-GAGCTTGCTTTCCTGATTGC-3'
E-cadherin	5'-ACTTTGGTGTGGGTCTGGAG-3'	5'-CAGGAGAAGAGTCCCTGTGC-3'
Wnt4	5'-CTCAAAGGCCTGATCCAGAG-3'	5'-TCACAGCCACACTTCTCCAG-3'
Wnt7b	5'-CAGGCAGAAAGGTTCTGGAG-3'	5'-GTCCTCCTCGCAGTAGTTGG-3'
Fzd2	5'-ATCATCTTCCTGTCCGGTTG-3'	5'-GAACCAGGTGAGGGACAGAA-3'
Galectin-3	5'-GCTGATTTCCCTGAGGTTCT3'	5'-CGACATCGCCT TCCACTTT-3'
GAPDH	5'-CACCACCATGGAGAAGGC-3'	5'-CCATCCACAGTCTTCTGA-3'

Mattos et al., 2014.

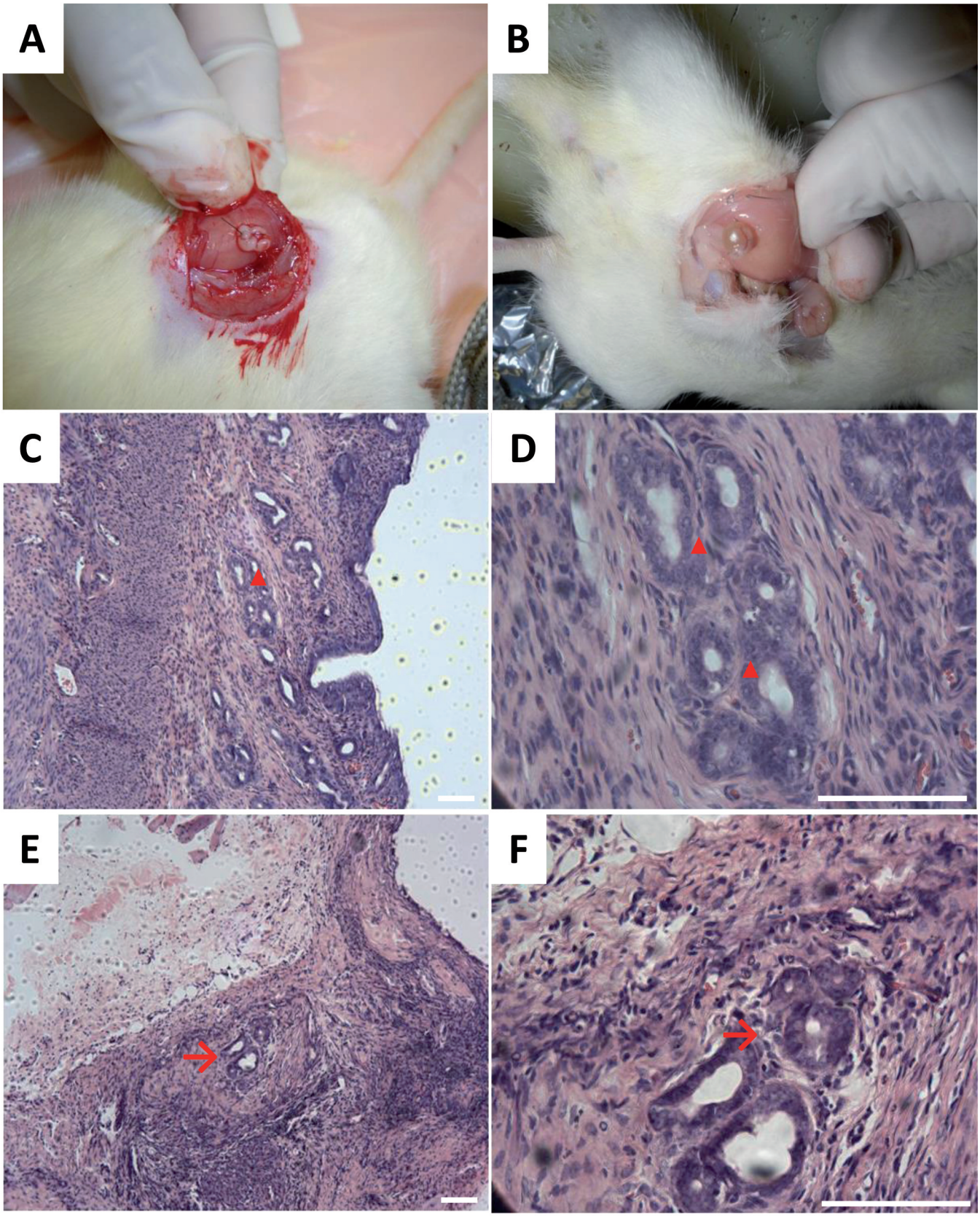


Fig. 1. Morphological characterization of endometriotic lesions. **A.** Macroscopic view of implants in the peritoneal cavity wall at the moment of implantation. **B.** Macroscopic view of implants 30 days after surgery when endometriotic lesions developed and showed total adherence to the peritoneal cavity wall. **C and D.** Photomicrographs of HE-stained histological sections of eutopic endometrium. **E and F.** Photomicrographs of HE-stained histological sections of peritoneal endometriosis; (arrowhead) Glandular structures in the endometrial stroma; (arrow) Endometriotic glands; Scale bars: 100 µm.

Results

Ectopic endometrium exhibits altered expression of Wnt-pathway genes

We performed quantitative qRT-PCR to evaluate possible differences in the Wnt-pathway gene expression profile. Our results revealed, in endometriotic lesions compared to eutopic endometrium, significantly lower expressions of the epithelial cell marker *E-cadherin*, and glycogen synthase kinase-3β (*Gsk3β*), a component of the β-catenin degradation complex. Moreover, our analysis of endometriotic lesions also revealed a significant increase in mRNA expression of *Wnt4* and *Wnt7b*, both of which are Wnt ligands and pathway activators. However, we could not detect differences in the expression of *Fzd2*, a Wnt receptor, and of *β-catenin*, the effector of the canonical Wnt pathway (Fig. 2A). We performed Western Blot analyses and showed that GSK3β protein expression is lower in the ectopic endometrium (Fig. 2B), whereas β-catenin protein expression is similar in both tissues (Fig. 2C).

β-catenin migrates to cell nuclei in the ectopic endometrium

Activation of the canonical Wnt/β-catenin signaling pathway involves the accumulation and stabilization of cytosolic unphosphorylated β-catenin, which then translocates to the nucleus. The nuclear localization of free β-catenin is a hallmark of this pathway activation (Van Noort et al., 2002). Thus, we next evaluated the intracellular localization of β-catenin in eutopic and ectopic endometria. Our Western Blot results revealed that β-catenin expression is more pronounced in the nuclear fraction of ectopic compared to normal tissue

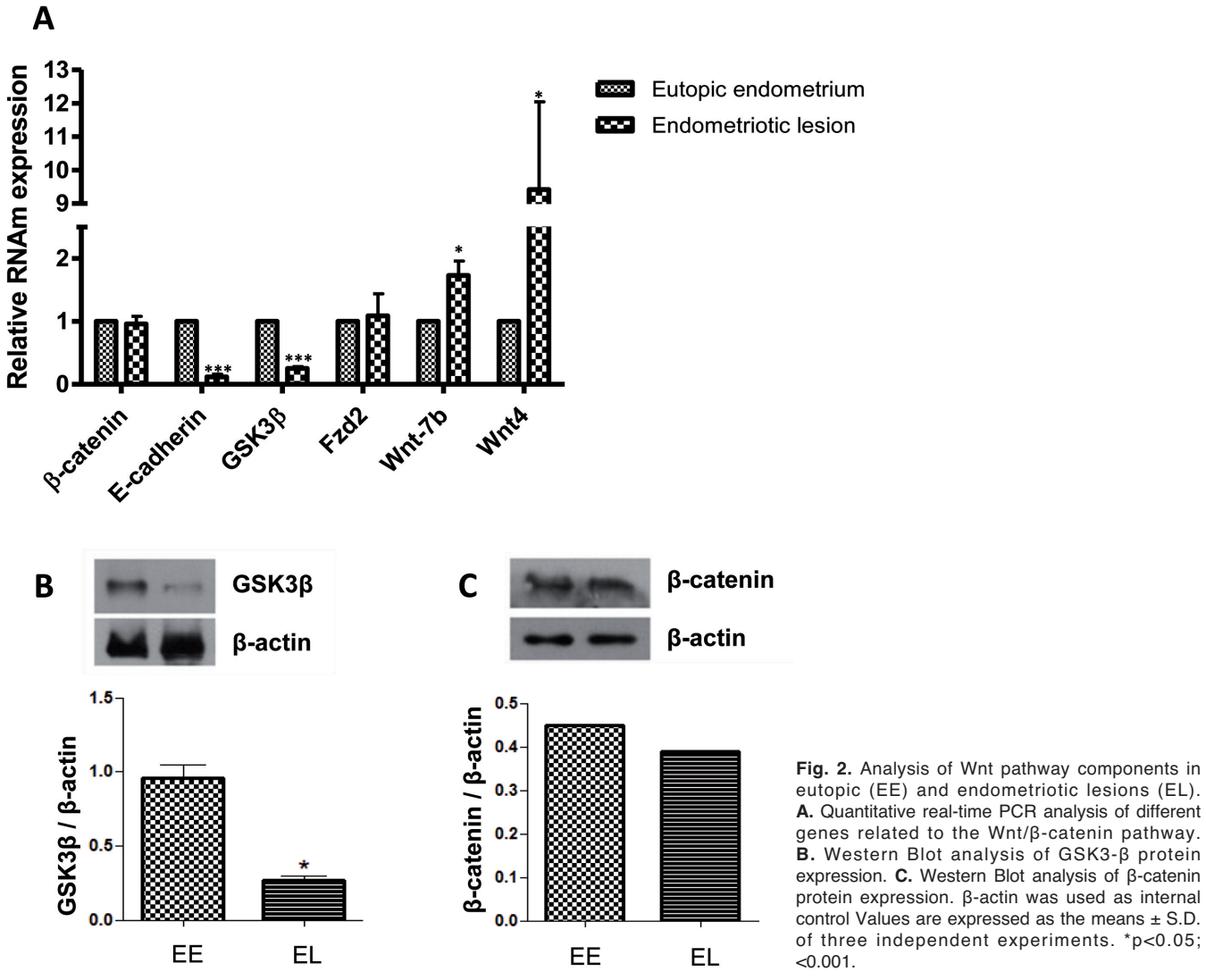
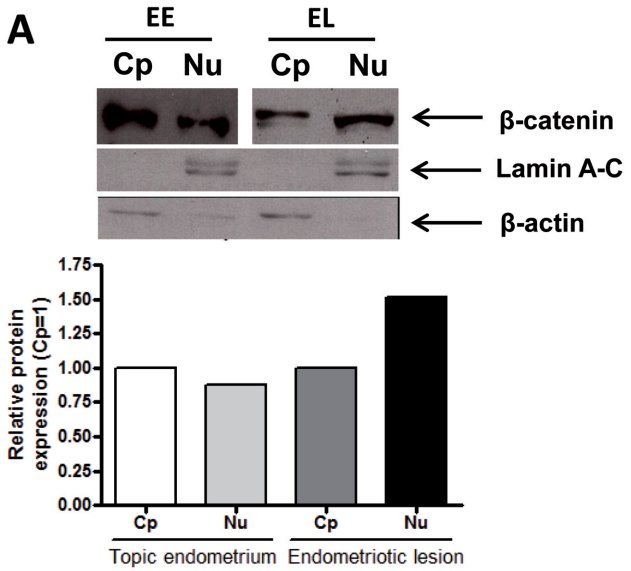


Fig. 2. Analysis of Wnt pathway components in eutopic (EE) and endometriotic lesions (EL). **A.** Quantitative real-time PCR analysis of different genes related to the Wnt/β-catenin pathway. **B.** Western Blot analysis of GSK3-β protein expression. **C.** Western Blot analysis of β-catenin protein expression. β-actin was used as internal control Values are expressed as the means ± S.D. of three independent experiments. *p<0.05; <0.001.

(Fig. 3A). These findings are confirmed by immunofluorescence analysis of both tissues. Epithelial β-catenin immunostaining was positive in eutopic and ectopic tissues (Fig. 3B,C). In stromal cells, immunostaining was positive in only 45.4% of the samples of eutopical endometrial tissue, while in ectopic endometrial tissue this same staining was observed in 77.8% of cases. Moreover, nuclear β-catenin localization was found in 33.3% and 18.2% of ectopic and eutopic tissues, respectively.



Galectin-3, an important regulator of the Wnt pathway, is overexpressed in endometriosis

Galectin-3 (Gal-3) participates in the Wnt pathway regulation, at least in part, by translocating β-catenin into the nucleus, thus promoting the transcriptional activation of target genes (Elad-Sfadia et al., 2004; Shimura et al., 2004, 2005; Shi et al., 2007; Song et al., 2009). In order to evaluate if the expression of this molecule is modulated in endometriosis we analyzed both, gene and protein expression of Gal-3. Our results showed higher mRNA and protein levels in ectopic lesions compared to eutopic endometrium tissue (Fig. 4A,B).

Discussion

The Wnt/β-catenin signaling pathway plays an important role in tumor development, especially colon tumors (Polakis, 1999; Barker and Clevers, 2006; Holcombe et al., 2002). This pathway is directly associated with pathophysiological events that drive tumor growth such as cell proliferation, invasion and the formation of an abundant blood supply through angiogenesis (Zhang et al., 2001; Clevers, 2006;

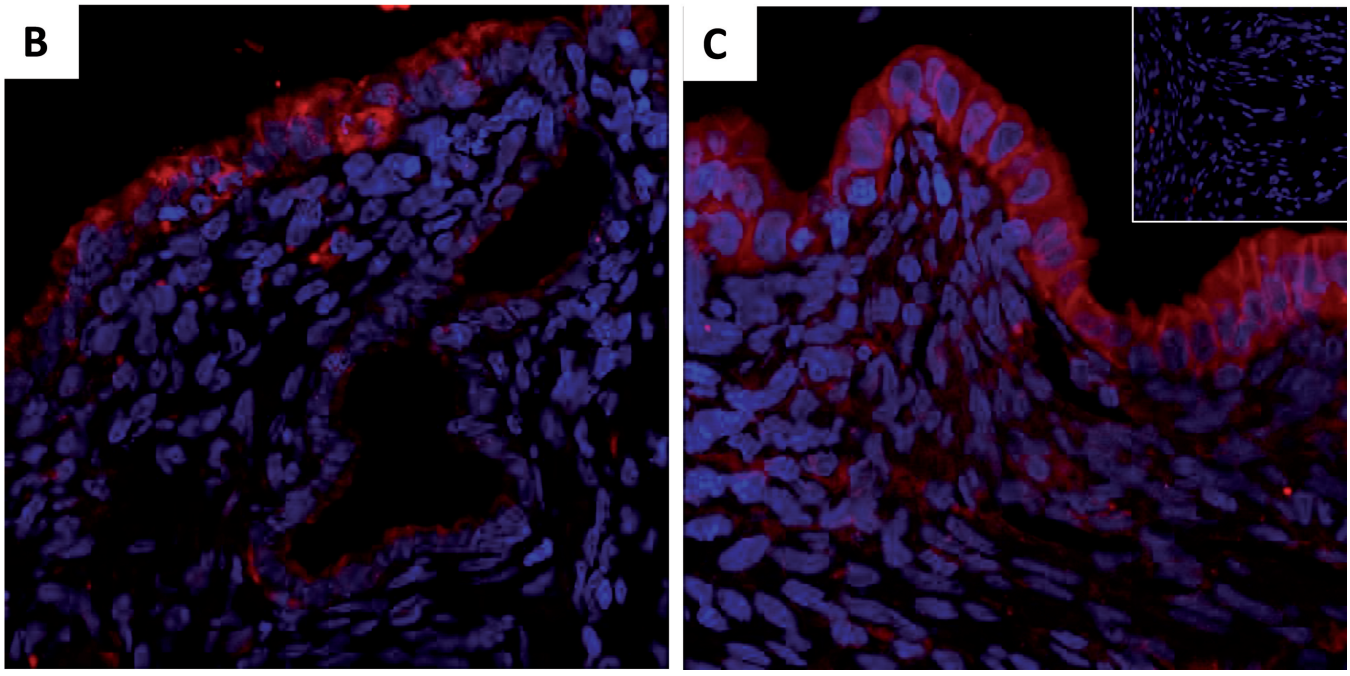


Fig. 3. β-catenin distribution in eutopic endometria and endometriotic lesions. **A.** Western Blot analysis of the β-catenin expression in cytoplasmic (Cp) and nuclear (Nu) extracts from eutopic endometria (EE) and endometriotic lesions (EL). β-actin was used as internal control. **B.** Photomicrograph of a eutopic endometrium stained for β-catenin (red). **C.** Photomicrograph of an endometriotic lesion stained for β-catenin (red). DAPI (blue). Insert negative control. x 630

Norimatsu et al., 2007; Takemaru et al., 2008; Wang et al., 2009). All of these events have also been seen and have been widely studied in endometriosis, especially angiogenesis. Several works, including data of our group, demonstrated an important role of this process for the establishment, growth and maintenance of endometriotic lesions, through a higher expression of VEGF, its receptor Flk-1 (VEGFR2) and a greater vascular density (Machado et al., 2008, 2010a,b). Thus, the molecular changes related to this pathology may resemble those found in tumors. Therefore, in this study, we analyzed the expression of canonical Wnt pathway components to assess alterations that might play a role in the development and maintenance of endometriotic lesions.

Our results showed that *Wnt4* and *Wnt7b* ligands, molecules responsible for triggering pathway activation after their binding to a specific receptor, were more expressed in endometriotic lesions than in the eutopic endometrium. These results are in line with previous findings by Gaetje et al. (2007), which showed increased levels of Wnt7a mRNA in ectopic endometrium and suggested an association between activation of the canonical Wnt pathway and endometriotic lesions. There are several reports in the literature about the role of Wnt4 and Wnt7b during normal uterus development, including stromal differentiation and postnatal endometrial adenogenesis (Franco et al., 2011; Hayashi et al., 2011; Li et al., 2013). The importance of Wnt ligands in the development of endometriosis is still unclear, however there are some reports showing that alterations in Wnt4 gene, including polymorphisms, increase the susceptibility to the development of endometriotic lesions (Luong et al., 2013; Pagliardini et al., 2013; Lee et al., 2014; Liang et al., 2016; Mafra et al., 2015; Wu et al., 2015). Concerning the role of wnt7b in endometriosis, there are no reports in the literature. Meanwhile, studies of different tumors address the importance of this molecule, as well as Wnt4, in controlling proliferation, cell invasion and angiogenesis (Huguet et al., 1994; Lejeune et al., 1995; Bui et al., 1997; Yan et al., 2011). Furthermore, there is *in vitro* evidence showing that Wnt genes, including Wnt7b, are capable of causing partial transformation in the breast epithelial cell line C57MG (Wong et al., 1994) and embryonic fibroblasts C3H 1OT1/2 (Bradbury et al., 1994). These previous works, our current study, and the parallels between tumors and endometriosis shed light on the possible roles of Wnt genes in the ectopic endometrium.

Decreased GSK3β expression in endometriotic lesions constitutes another indication of canonical Wnt pathway activation found in our work. Because GSK3β degrades cytoplasmic β-catenin when the pathway is not activated (Cleavers, 2006), decreased expression of this enzyme indicates lower β-catenin degradation, which, in turn, allows its nuclear translocation and consequent transcriptional activation of target molecules (Reya and Clevers, 2005). Studies indicate that GSK3β inhibition favors cell growth (Piazza et al., 2010), inhibits cell death by apoptosis (Linseman et al., 2004; Maurer et al., 2006) and supports tumorigenesis (Korur et al., 2009). Furthermore, GSK3β in its regular active state promotes apoptosis and inhibits migration of endothelial cells towards VEGF and FGF, preventing the formation of capillaries. On the other hand, when the catalytic site of GSK3β is inhibited, these effects are reversed (Kim et al., 2002; Skurk et al., 2005). Since angiogenesis is a fundamental process in the development of endometriosis, and GSK3β negatively regulates this process, its low expression could be correlated with the prominent angiogenic process in endometriosis.

Our current study shows that *E-cadherin* transcripts are expressed to a lower level in ectopic than in eutopic endometrium. This result is in line with our observation that stromal tissue is much more abundant in developed endometriotic lesions than glandular epithelium. Both findings suggest a regression of endometrial glands in

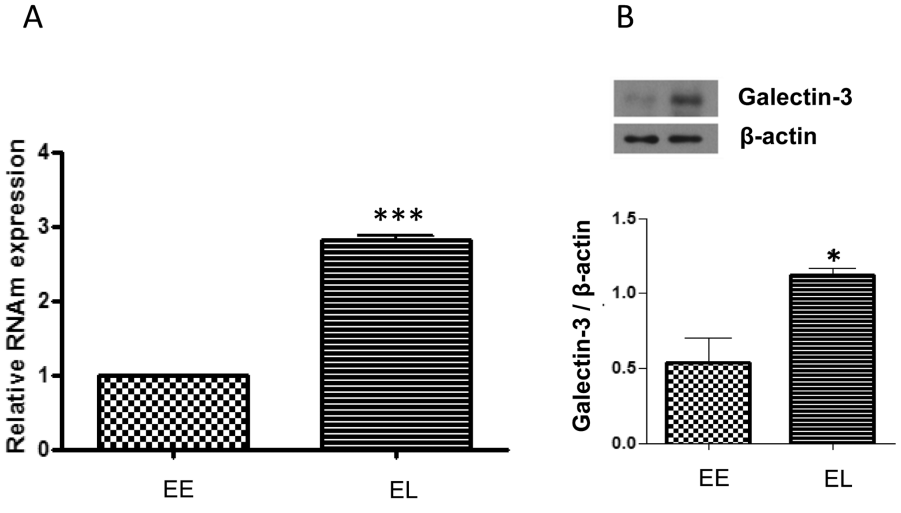


Fig. 4. Galectin-3 expression in eutopic endometrium (EE) and endometriotic lesions (EL). **A.** Quantitative real-time PCR analysis of Gal-3 genic expression. GAPDH mRNA was used as internal control. **B.** Western Blot analysis of Gal-3 protein expression. Values are expressed as the means ± S.D of three independent experiments. *p<0.05; ***p<0.001.

lesions compared with the eutopic endometrium. A possible explanation for this fact is the process of epithelial-mesenchymal transition which is associated to the pathogenesis of endometriosis and is promoted by the Wnt/β-catenin pathway (Kim et al., 2002; Skurk et al., 2005; Matsuzaki and Darcha, 2012). E-cadherin has been considered as a classical tumor suppressor gene, as its expression is reduced in some tumors, such as lobular breast carcinomas, and in various tumor cell lines such as cells from human gastric carcinomas (Hirohashi, 1998; Conacci-Sorrell et al., 2002). Therefore, the lower expression of *E-cadherin* in endometriotic lesions further supports the hypothesis that this disease and malignant tumors behave similarly.

In normal epithelial cells, most β-catenin forms a complex with E-cadherin and other molecules, contributing to cell-cell adhesion (Gottardi and Gumbiner, 2001). Our results showed lower E-cadherin expression in endometriotic lesions, and we would also expect to see lower levels of β-catenin, as the two molecules are so closely associated. However, the analysis of β-catenin expression revealed no significant differences between the lesions and the eutopic endometrium. Conacci-Sorrell et al., 2002, reported that the disruption of adherens junctions by the loss of E-cadherin releases β-catenin from the complex and, if not rapidly degraded, β-catenin accumulates in the cytoplasm and translocates to the nucleus, thus activating gene transcription. In the present study, β-catenin was located not only on the plasma membrane and cytoplasm, but also accumulated in the nuclei of endometriotic cells. Nuclear localization of β-catenin is the key step in Wnt/β-catenin signaling activation (MacDonald et al., 2009), so the translocation of β-catenin to cell nuclei provides further evidence of the activation and potential involvement of the Wnt/β-catenin pathway in endometriotic lesions. Indeed, the evaluation of phosphorylated beta-catenin is a useful way to demonstrate the activation of canonical Wnt pathway, since the non-phosphorylated beta-catenin accumulates in the cytoplasm and can migrate to the cell nucleus and activate transcription of several genes. However, the evaluation of nuclear beta-catenin shows the same process, since there are reports in the literature of this molecule is only free to migrate to the nucleus if it is not phosphorylated (Huang et al., 2015; Kraus et al., 2015; Li et al., 2015). Our results differ, however, from other studies showing no β-catenin expression in cell nuclei of human endometriotic tissues (Shaco-Levy et al., 2008; Van Patten et al., 2010).

Gal-3 has been shown to exert functions in the Wnt/β-catenin signaling pathway. Among these functions, Gal-3 promotes transcriptional activation by binding to β-catenin and TCF4 (Shimura et al., 2004, 2005), and it also induces PI3K/AKT-mediated phosphorylation and inhibition of GSK3β which, in turn, increases the levels of β-catenin (Song et al., 2009). Previous studies have also shown that Gal-3 inhibition decreases Wnt signaling and induces apoptosis (Shi et al., 2007). Here, we showed an increased expression of Gal-3 in endometriotic lesions. This result corroborates the work of Noël et al. (2011), which showed a higher expression of Gal-3 in human endometriotic lesions and in the eutopic endometrium of women with endometriosis. Together, these data indicate a higher activation of Wnt signaling in endometriotic lesions, and may explain the decreased expression of GSK3β and the increased nuclear localization of β-catenin observed in this study. Furthermore, other studies characterized Gal-3 as a pro-angiogenic molecule (Markowska et al., 2011; Wan et al., 2011), suggesting that the high expression of this molecule in endometriotic lesions could favor the onset and maintenance of this disease.

We conclude that the expression of Wnt/β-catenin pathway components analyzed in this study is significantly altered in endometriotic lesions. Our data indicate an increased activity of this signaling pathway in endometriosis, which could be directly related to typical characteristics of this pathology, such as increased cell proliferation and prominent angiogenic activity. Further understanding of the role played by this pathway in the onset and maintenance of endometriosis may guide the study of targeted therapies.

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