NF-κB and COX-2 Expression in Nonmalignant Endometrial Lesions and Cancer

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ABSTRACT

Objectives: *To examine the immunohistochemical expression of cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) in benign endometrial polyps (EPs), endometrial hyperplasia (EH), endometrial intraepithelial neoplasia (EIN), and endometrioid endometrial cancer (EC).*

Methods: *The immunohistochemical expression of COX-2 and NF-κB was performed using an Aperio Scanscope XT automated system in 218 patients with endometrioid EC and 107 patients with nonmalignant endometrial lesions: 53 with benign EPs, 37 with EH, and 17 with EIN.*

Results: *COX-2 and NF-κB p50 expression were significantly lower in EC compared with nonmalignant lesions. We observed significant decreased NF-κB p65 expression in EC vs EPs (*$P < .001$*) and EH (*$P = .014$*) as well as in EIN vs EPs (*$P = .01$*). For patients with EC, COX-2 correlated positively with NF-κB p65 and NF-κB p50 (*$P < .001$*). Grade 3 tumors had a higher mean expression of NF-κB p65 (*$P = .03$*). NF-κB p50, NF-κB p65, and COX-2 expression had no impact on survival.*

Conclusions: *We conclude that COX-2 and NF-κB expression are lower in EC compared with nonmalignant endometrial lesions. COX-2 and NF-κB expression have no prognostic value in EC.*

Endometrial cancer (EC) is the most common cancer of the female genital tract in developed countries.[1] It develops most commonly in postmenopausal women and usually presents as an early-stage disease.[2] The currently accepted dual model of carcinogenesis categorizes EC as type 1 or type 2 based on genetic and morphologic features.[3,4]

Continuous stimuli with nonopposed estrogen results in endometrial hyperplasia (EH), which can progress to a more complex architecture that is associated with cytologic atypia. Further, type 1 EC can develop from atypical hyperplastic lesions.[5]

Certain cell physiologic changes are necessary to effect the transformation of EH into a malignant phenotype. Many such alterations have been described in type 1 EC, such as microsatellite instability, PTEN inactivation, *KRAS* mutations, and mutations in β-catenin.[6]

Cyclooxygenase-2 (COX-2) converts arachidonic acid in the cytoplasmic membrane into prostaglandin H2 and, subsequently, to prostaglandin E2 (PGE2). PGE2 regulates cell proliferation, differentiation, and apoptosis through several autocrine and paracrine pathways. The most significant pathways that link PGE2 and cancer are the Ras-ErK, GSK3β-β-catenin, PI3K-AKT, BCL2, SRC-EGFR, and nuclear factor-κB (NF-κB) pathways.[7]

NF-κB is a family of transcription factors that comprises five subunits: p50, p52, c-Rel, RelB, and p65. In the classic, or canonical, pathway, NF-κB is the chief heterodimer, consisting of p50 and p65. The NF-κB complex is rendered inactive in the cytoplasm by inhibition of κB (IκB). The classic NF-κB pathway is activated on phosphorylation of IκB by the IκB kinase complex. NF-κB is then released and translocates to the nucleus, where it binds to κB-responsive elements in NF-κB target genes.[8,9]

NF-κB and COX-2 can be stimulated directly by estrogen receptor, and NF-κB interacts with COX-2 to regulate transcription.[10,11] This interaction occurs directly—the human COX-2 promoter contains a binding site for NF-κB.[12,13] Moreover, accumulating evidence suggests that NF-κB promotes the development and progression of cancer.[8,9,14] Increased NF-κB signaling can block apoptosis, increase proliferation, and induce epithelial-mesenchymal transition[14] by activating NF-κB target genes. Thus, the interaction between the NF-κB and COX-2 signaling pathways may constitute an important pathogenic mechanism of the carcinogenesis of type 1 (endometrioid) EC.

We hypothesized that in a multistep model of the carcinogenesis of endometrioid EC, the activation of COX-2 and NF-κB signaling mediates the progression of hyperplasia to cancer. Our aim was to retrospectively evaluate the immunohistochemical expression of NF-κB and COX-2 in cases of benign endometrial polyps (EPs), EH, endometrial intraepithelial neoplasia (EIN), and endometrioid EC. We also evaluated the prognostic value of NF-κB and COX-2 in EC.

Materials and Methods

Patient Characteristics

EIN nomenclature was used.[15] Hyperplasia without atypia was considered as EH, and hyperplasia with atypia as EIN.

This retrospective analysis included a series of 218 patients with EC and 107 patients with nonmalignant disease—53 with benign EPs, 37 with EH, and 17 with EIN—who were admitted to the Department of Gynecologic Oncology, AC Camargo Cancer Hospital (São Paulo, Brazil) from January 1990 to December 2008.

Clinical and pathologic data were obtained from the medical records. Patients with EC were staged based on the International Federation of Gynecology and Obstetrics (FIGO), 2009. One hundred sixty-one (73.9%) were stage I, 11 (5%) were stage II, 37 (17%) were stage III, and 9 (4.1%) were stage IV. Fifty-one (23.4%) had grade 3 (FIGO) tumors, and 89 (40.8%) patients had deep myometrial invasion (>50%).

Tissue Microarray Construction (TMA)

H&E-stained sections of nonmalignant specimens and EC cases were reviewed, and the areas of the tumors were marked on the slides. From each paraffin block, one tissue core (1 mm in diameter) was sampled from each marked area in the donor block and mounted on a recipient paraffin block using a custom-made instrument (Beecher Instruments, Silver Springs, MD). In the resulting block, the tissue cylinders were aligned and marked for identification on a chart. Cores were spaced 0.2 mm apart.

Immunohistochemical Staining

Three-micrometer TMA sections were transferred to an adhesive-coated slide system (Instrumentics Inc, Hackensack, NJ), and antigen was detected by a streptavidin-biotin-peroxidase technique (StreptABC, Dako, Carpinteria, CA). The reactions were accompanied by a positive control and two negative controls—one that lacked the primary antibody and another that lacked the secondary antibody.

Rabbit polyclonal antihuman NF-κB p65 (RB-9034P1, dilution 1:100) and rabbit polyclonal antihuman NF-κB p50 (RB-1648P1, dilution 1:50) were purchased from Neomarkers (Fremont, CA), and anti–COX-2 (clone NCL-COX-2, dilution 1:1000) was purchased from Novocastra Laboratories (Newcastle, UK).

The primary antibodies for NF-κB and COX-2 generated granular or diffuse cytoplasmic staining. The cytoplasmic immunostaining was evaluated quantitatively. No evidence of nuclear expression of NF-κB or COX-2 was found. Immunohistochemical expression of NF-κB (p50 and p65) and COX-2 was analyzed using an automated computer system with positive pixel count. Briefly, the slides were placed in an Aperio Scanscope XT system (Aperio, Vista, CA). A robotic microscope scanned each slide, and the Aperio captured images from each slide, quantified the staining intensity in a selected region, and calculated a numeric score. The operator quantified at least six areas with the highest staining intensity. Two pathologists (I.W.C. and C.A.B.T.O.) analyzed all slides. Data on the original molecular markers were collected as continuous variables ranging from 0 to 100. **Image 1** shows examples of NF-κB p50, NF-κB p65, and COX-2 expression.

Statistical Analysis

The database was generated using SPSS, version 16.0 for Mac (SPSS, Chicago, IL) and MedCalc, version 11 (MedCalc, Ostend, Belgium). The sample was characterized by descriptive statistics. We compared quantitative variables between groups using the Mann-Whitney or Kruskal-Wallis test. We performed extensive post hoc multiple comparisons using the Mann-Whitney test with Bonferroni correction for the significance level. Spearman coefficient was used to analyze the correlation between numeric and ordinal values. We compared the cytoplasmic expression of markers between the following groups: EP, EH, EIN, and EC.

For patients with EC, progression-free survival was defined as the time from surgery to the date of recurrence or last follow-up. Overall survival was defined as the time from surgery to the date of death or last follow-up. For progression-free survival analysis, patients with stage IV disease were excluded. For progression-free and overall survival analysis, we excluded patients admitted after 2005 because all patients should have the chance to be followed up for at least 5 years. Therefore, 144 patients were included in progression-free

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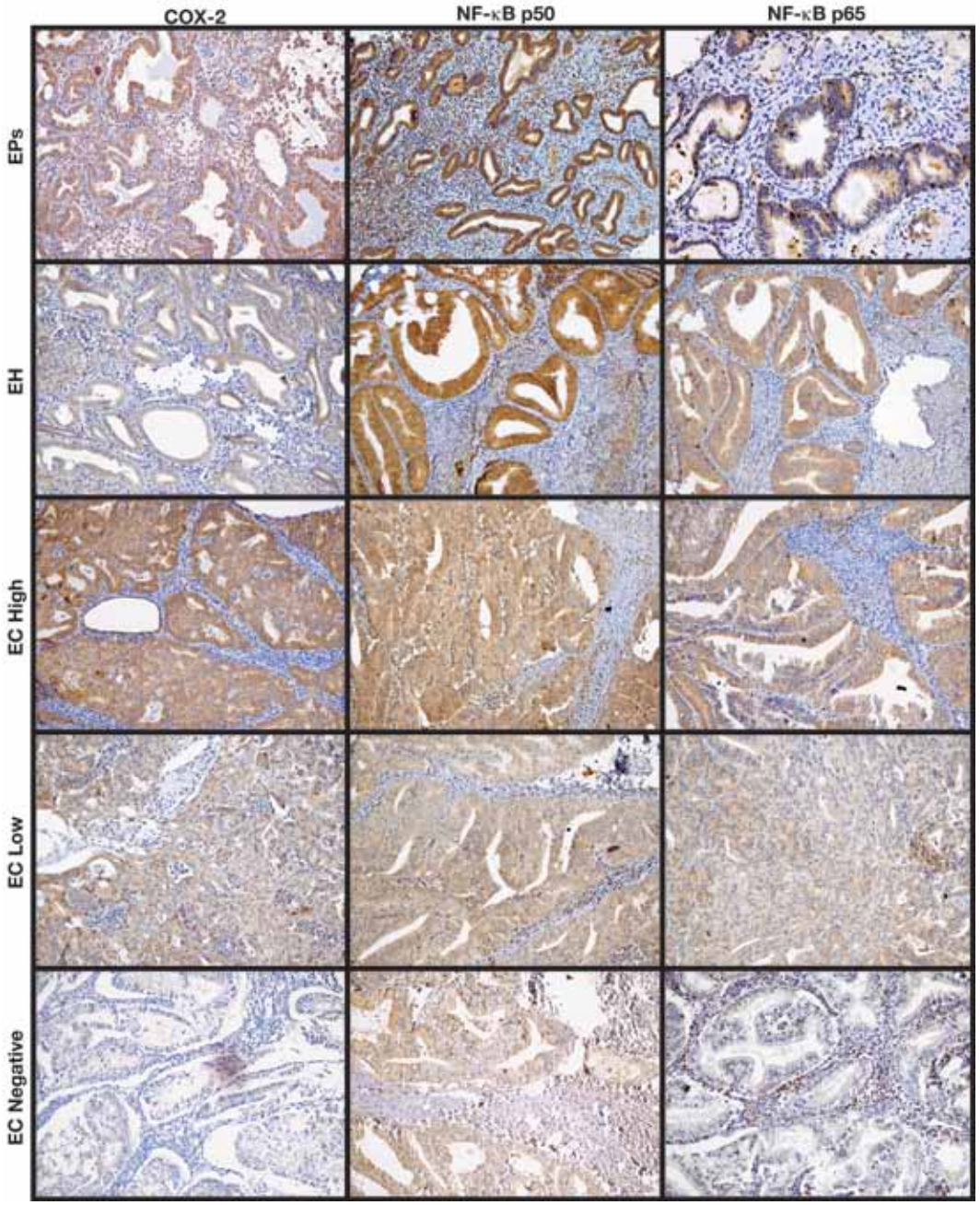

Image 1 Examples of cyclooxygenase-2 (COX-2), nuclear factor-κB (NF-κB) p50, and NF-κB p65 expression. EC, endometrial cancer; EH, endometrial hyperplasia; EPs, endometrial polyps.

survival and 165 in overall survival analysis. Survival curves were constructed by Kaplan-Meier life table analysis and compared with a log-rank test.

Information on the original molecular markers was collected as continuous variables ranging from 0 to 100. For survival analysis, the continuous variables were converted into dichotomic variables (low and high expression). The cutoff point was determined by receiver operating characteristic curve and the reference based on the occurrence of death during follow-up. The cutoff points for NF-κB p50, NF-κB p65, and COX-2 expression were 51, 74, and 40, respectively. In all tests, the level of significance was 5%.

Results

The mean (standard deviation [SD]) immunohistochemical expression scores for COX-2 in EPs, EH, EIN, and EC were 73.9 (19.3), 79.9 (19.1), 73.6 (13.3), and 55.1 (24.1), respectively **Table 1**. Post hoc analysis found no significant difference in COX-2 expression between EH and EIN, but

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Table 1
Descriptive Analysis of COX-2, NF-κB p50, and NF-κB p65 Immunohistochemical Expression Based on Endometrial Lesion Type

Endometrial Lesion Type	No. of Patients	Mean Expression	Standard Deviation	Minimum	Maximum
Polyps					
COX-2	52[a]	66.01	19.28	16.65	91.45
NF-κB p50	50[b]	85.14	13.74	46.05	99.08
NF-κB p65	53	77.78	14.04	30.48	93.30
Endometrial hyperplasia					
COX-2	35[c]	73.97	19.13	0.37	91.02
NF-κB p50	35[c]	80.50	19.94	19.56	99.22
NF-κB p65	36[a]	73.41	21.60	0.52	96.07
Endometrial intraepithelial neoplasia					
COX-2	15[c]	70.05	13.32	41.43	86.02
NF-κB p50	17	82.73	10.76	60.50	98.58
NF-κB p65	16[a]	67.80	16.48	33.48	85.95
Endometrial cancer					
COX-2	213[d]	51.75	24.07	1.62	96.57
NF-κB p50	213[d]	58.63	21.53	2.30	95.83
NF-κB p65	218	64.55	22.07	2.34	97.62

COX-2, cyclooxygenase-2; NF-κB, nuclear factor-κB.
[a] One case excluded.
[b] Three cases excluded.
[c] Two cases excluded.
[d] Five cases excluded.

expression of COX-2 was significantly lower in EC compared with nonmalignant lesions. COX-2 expression was higher in EH than in EPs ($P = .004$) **Table 2**.

The mean (SD) immunohistochemical expression scores for NF-κB p50 in EPs, EH, EIN, and EC were 90.1 (13.7), 87.3 (19.9), 85.3 (10.8), and 61.2 (21.5), respectively (Table 1). We found no significant difference in mean NF-κB p50 expression between nonmalignant endometrial lesions. NF-κB p50 expression was lower in EC compared with nonmalignant lesions ($P < .001$) (Table 2).

The mean (SD) immunohistochemical expression scores for NF-κB p65 in EPs, EH, EIN, and EC were 81.5 (14.0), 81.4 (21.6), 73.6 (16.5), and 70.5 (22.1), respectively (Table 1). NF-κB p65 expression was significantly lower in EC compared with EPs ($P < .001$) and EH ($P = .014$), as well as in EIN vs EPs ($P = .01$) (Table 2).

COX-2 correlated positively with NF-κB p65 (rs = 0.654; $P < .001$) and NF-κB p50 (rs = 0.611; $P < .001$). We also noted a link between NF-κB p65 and NF-κB p50 (rs = 0.592; $P < .001$) **Table 3**.

Across cases of EC, there was no correlation between depth of invasion and expression of COX-2 ($P = .77$), NF-κB p65 ($P = .98$), or NF-κB p50 ($P = .77$). Similarly, histologic grade was not associated with expression of COX-2 ($P = .21$) or NF-κB p50 ($P = .08$). However, grade 3 tumors had a higher mean expression of NF-κB p65 ($P = .03$), and a higher body mass index was associated with a higher expression of NF-κB p50 ($P = .026$) **Table 4**.

The 5-year progression-free and overall survival rates for women with EC were 80.6% and 78.2%, respectively. NF-κB p50, NF-κB p65, and COX-2 expression had no effect on both progression-free and overall survival. **Table 5** and **Table 6**

Table 2
Multiple Comparisons of the Immunohistochemical Mean Expression of COX-2, NF-κB p50, and NF-κB p65 Based on Endometrial Lesion Type[a]

Endometrial Lesion Type	Endometrial Hyperplasia, *P*	Endometrial Intraepithelial Neoplasia, *P*	Endometrial Cancer, *P*
Polyps			
COX-2	.004	.609	<.001
NF-κB p50	.486	.176	<.001
NF-κB p65	.616	.010	<.001
Endometrial hyperplasia			
COX-2		.097	<.001
NF-κB p50		.592	<.001
NF-κB p65		.054	.014
Endometrial intraepithelial neoplasia			
COX-2			.002
NF-κB p50			<.001
NF-κB p65			.786

COX-2, cyclooxygenase-2; NF-κB, nuclear factor-κB.
[a] Bonferroni α = 0.83%.

Table 3
Correlation Between Mean Expressions of COX-2, NF-κB p65, and NF-κB p50 in Endometrial Cancer

	COX-2	NF-κB p65	NF-κB p50
COX-2			
ρ	1.000	0.654	0.611
P		<.001	<.001
No. of cases	213	213	211
NF-κB p65			
ρ	0.654	1.000	0.592
P	<.001		<.001
No. of cases	213	218	213
NF-κB p50			
ρ	0.611	0.592	1.000
P	<.001	<.001	
No. of cases	211	213	213

COX-2, cyclooxygenase-2; NF-κB, nuclear factor-κB.

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Table 4
Mean NF-κB p50, NF-κB p65, and COX-2 Expression Based on Clinicopathologic Characteristics of Patients With Endometrioid Endometrial Cancer

	COX-2			NF-κB p50			NF-κB p65		
Variable	**No.**	**Mean Expression**	***P***	**No.**	**Mean Expression**	***P***	**No.**	**Mean Expression**	***P***
Age (continuous variable)	213	—	.42	213	—	.16	218	—	.41
Body mass index (continuous variable)	179	—	.26	180	—	.026	182	—	.41
Tumor size									.94
<2 cm	47	57.84	.58	46	60.53	.78	47	72.01	
≥2 cm	149	50.85		148	58.71		152	63.47	
Myometrial invasion									.98
≤50%	122	53.86	.08	121	59.06	.73	124	64.42	
>50%	86	47.62		87	57.22		89	64.26	
Architectural grade									.033
G1	97	50.23	.20	95	56.29	.084	98	63.75	
G2	81	51.12		83	58.86		84	62.02	
G3	15	59.13		25	66.89		26	73.39	
Lymphatic space invasion									.12
Absent	163	50.73	0.51	163	50.73	0.51	168	62.96	
Present	32	54.47		32	54.47		32	69.90	
Pelvic lymph node involvement									.77
Yes	15	48.39	0.69	15	67.07	0.086	15	64.07	
No	196	51.68		196	57.63		201	64.34	

COX-2, cyclooxygenase-2; G, grade; NF-κB, nuclear factor-κB.

summarize the association between clinicopathologic variables and, respectively, progression-free and overall survival.

Discussion

The function of COX-2 in carcinogenesis has been examined in colorectal cancer and as a prognostic marker for ovarian, breast, and gastric cancer.[16-20] Yet, in several cancers, including breast, prostate, skin, lung, and pancreas, NF-κB expression is linked to poorer clinical outcome.[21-25] However, the immunohistochemical expression of NF-κB has not been examined in EPs, EH, and EIN.

Scoring systems for tumor markers are usually based on the proportion of positive tumor cells and staining intensity.[26] However, the interpretation of staining intensity is highly subjective and can be affected by storage time and variations in protocols and fixation procedures. Computer-assisted automated analysis programs help eliminate the inherent variability in pathologist-based scores and can increase the sensitivity of measurements of protein expression.[27] Thus, we chose to use an automated system in our study.

Type 1 EC (endometrioid histology) constitutes the majority of EC cases and is associated primarily with estrogen stimulation.[2] The classic steroid signaling pathway entails the activation of estrogen-responsive elements in the genome by estrogen receptor.[28,29]

A secondary mechanism steroid signaling involves protein-to-protein interactions,[28,29] wherein a receptor and ligand activate transcription factors, such as NF-κB, activator protein 1, and specificity protein 1, which subsequently stimulate transcription.[30-33] In addition, estrogen correlates with inflammatory environments, stimulating the expression of inflammatory cytokines, such as interleukin (IL) 1, tumor necrosis factor α, matrix metallopeptidases, and IL-1b.[34,35] A direct interaction between NF-κB and COX-2 transcription has also been proposed[10,11]—the COX-2 promoter contains a binding site for NF-κB.[12,13]

Mizumoto et al[36] recently demonstrated that, despite its central role in endometrial carcinogenesis, the conventional KRAS-ERK1/2 pathway is insufficient to effect endometrial carcinogenesis and that NF-κB is a critical target of *KRAS*-induced endometrial carcinogenesis.

We noted significant differences in mean COX-2 expression in various nonmalignant lesions (EPs, EH) and EC. Mean COX-2 expression decreased progressively from EH to EIN and EC. In our series, we observed higher expression of COX-2 in EH than in EPs. Although polyps are not premalignant lesions, COX-2 expression might be attributed to the intense inflammatory process during its pathogenesis. This hypothesis has been corroborated by Cicinelli et al,[37] who reported in 96 cases that COX-2 expression rose in 93.7% of small polyps with signs of chronic endometritis. Interestingly, COX-2 expression may differ according to the menopausal status, as reported by Erdemoglu et al,[38] who found significantly higher expression in premenopausal polyps compared with postmenopausal polyps.

Instead of analyzing the normal endometrium, we opted to include benign polyps because polyps are benign tumors but not premalignant. Only a few authors have analyzed COX-2 expression in both nonneoplastic endometrium and cancer. Erkanli et al[39] evaluated 50 ECs, 30 simple hyperplasias, and 20 proliferative endometria and found

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Table 5
PFS Rates of 144 Patients Based on Clinicopathologic Variables and NF-κB p50, NF-κB p65, and COX-2

Variable and Category	No.	5-year PFS (%)	*P*
COX expression[a]			.636
Low	47	83.0	
High	93	79.6	
NF-κB p65 expression			.314
Low	88	83.0	
High	56	76.8	
NF-κB p50 expression			.117
Low	65	86.2	
High	75	76.0	
Myometrial invasion			.003
Restricted to the endometrium	20	95.0	
≤50%	66	86.4	
>50%	49	71.4	
Serosa invasion	8	50.0	
Myometrial invasion (grouped)			
≤50%	86	88.4	.002
>50%	57	68.4	
Histologic grade (FIGO)			.004
G1+G2	106	84.9	
G3	32	62.5	
Lymphatic space invasion			<.001
Absent	116	84.5	
Present	18	50.0	
Peritoneal cytology			
Positive	13	61.5	.080
Negative	83	83.1	
Undetermined	11	90.9	
Pelvic lymph node involvement			<.001
Yes	8	37.5	
No	136	83.1	
Para-aortic lymph node involvement			.001
Yes	5	40.0	
No	139	82.0	
Parametrial involvement			.003
Yes	5	40.0	
No	132	83.3	

COX-2, cyclooxygenase-2; FIGO, International Federation of Gynecology and Obstetrics; G, grade; NF-κB, nuclear factor-κB; PFS, progression-free survival.
[a] COX-2 mean expression cutoff points were 51, 74, and 40, respectively.

Table 6
OS Rates of 165 Patients Based on Clinicopathologic Variables and NF-κB p50, NF-κB p65, and COX-2

Variable and Category	No.	5-year OS (%)	*P*
COX expression[a]			.267
Low	55	83.6	
High	106	75.5	
NF-κB p65 expression			.076
Low	98	82.7	
High	67	71.6	
NF-κB p50 expression			.093
Low	72	84.7	
High	88	73.9	
Myometrial invasion			<.001
Restricted to the endometrium	20	100.0	
≤50%	72	87.5	
>50%	57	71.9	
Serosa invasion	13	23.1	
Myometrial invasion (grouped)			<.001
≤50%	92	90.2	
>50%	70	62.9	
Histologic grade (FIGO)			.013
G1+G2	117	82.1	
G3	40	65.0	
Lymphatic space invasion			<.001
Absent	124	85.5	
Present	26	38.5	
Peritoneal cytology			<.001
Positive	19	42.1	
Negative	90	83.3	
Undetermined	12	91.7	
Pelvic lymph node involvement			<.001
Yes	10	30.0	
No	154	81.2	
Para-aortic lymph node involvement			.219
Yes	5	60.0	
No	159	78.6	
Parametrial involvement			<.001
Yes	9	33.3	
No	143	84.7	

COX-2, cyclooxygenase-2; FIGO, International Federation of Gynecology and Obstetrics; G, grade; NF-κB, nuclear factor-κB; OS, overall survival.
[a] COX-2 mean expression cutoff points were 51, 74, and 40, respectively.

higher expression of COX-2 in EC and EH compared with proliferative endometrium. Similarly, Cao et al[40] suggested that COX-2 was not expressed in the non-neoplastic endometrium, minimally expressed in the grade 1 endometrioid carcinomas, and stained most strongly in the grade 2 and grade 3 endometrioid carcinomas. Conversely, Orejuela et al[41] did not find a statistically significant difference in COX-2 expression among EC (n = 14), all types of EH (n = 19), and normal endometrium (n = 10). However, the small number of patients may have impaired the results of that study.

We did not observe any correlation between COX-2 expression in EC and depth of invasion and histologic grade, for which conflicting data exist. Ferrandina et al[42] reported that well-differentiated tumors with superficial myometrial invasion had lower expression of COX-2 compared with high-grade tumors or deep myometrial invasion. Fowler et al[43] and Lambropoulou et al[44] also found that COX-2 expression correlated with higher histologic grade. The last three studies included nonendometrioid histology in their analysis. Similarly to our study, Erkanli et al[39] and Jeon et al[45] did not find any correlation between COX-2 expression and depth of invasion or histologic grade.

The prognostic value of COX-2 expression in EC is controversial. We did not find COX-2 expression to be a prognostic factor, in contrast with Lambropoulou et al,[44] who suggested that COX-2 expression was a prognostic factor but only in univariate analysis. Our findings corroborate with most studies, in which COX-2 expression had no effect on prognosis.[39,42,43,45]

Regarding the prognostic value of NF-κB p50 and NF-κB p65, we also did not find any correlation with progression-free and overall survival. As far as we know, our study is the first to analyze NF-κB expression as a prognostic factor in EC. Interestingly, we also found that grade 3 tumors had a higher

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mean expression of NF-κB p65, and higher body mass index was associated with higher expression of NF-κB p50.

The decreased immunohistochemical expression of COX-2 in EC vs nonmalignant lesions might be explained by the presence of microsatellite instability (MSI). COX-2 expression can be suppressed when there is a defect in mismatch repair genes and MSI.[46] Moreover, MSI appears to occur late in EC carcinogenesis—it is usually present in type 1 EC and is infrequent in EPs and EH without atypia.[47,48] Further studies should be performed to confirm this hypothesis because we did not evaluate MSI in our series.

Our data also demonstrate greater expression of NF-κB p50 and NF-κB p65 in nonmalignant endometrial lesions compared with EC. We noted a significant and positive correlation between mean expression of NF-κB and COX-2 in EC, indicating that when mean COX-2 expression decreases, NF-κB also declines.

During endometrial carcinogenesis, endometrial tissue is exposed to various stimuli, and proliferative changes occur over time. Certain nonmalignant and premalignant lesions have more intense inflammatory microenvironments, some of which can undergo progressive alterations in histologic complexity and atypia, which culminate in EC.[5,49]

The oncogenic and signal transduction pathways in endometrial carcinogenesis remain unknown,[36] and the reason why COX-2 and NF-κB expression decline in EC compared with nonmalignant lesions is undetermined. However, COX-2 and NF-κB are clearly expressed in premalignant lesions (hyperplasia) and EC; thus, this signaling pathway merits further examination in endometrial carcinogenesis. New information regarding the molecular mechanisms of endometrial carcinogenesis can guide the future development of therapeutics for cancer by suppressing the carcinogenic pathway.

The heterogeneity of the studies and their methods make any comparison difficult, and it is important to note that we used TMA instead of whole tissue section for immunohistochemical staining. Among other studies, only Fowler et al[43] also used TMA; however, they used a 0.6-mm tissue core while we used a 1-mm tissue core. Although it is argued that a single core sample per tumor may not be representative of the whole tumor, results using even one sample tumor have suggested an association between molecular features and clinicopathologic variables.[50,51]

In contrast to other studies, we performed a novel technique of COX-2 and NF-κB immunohistochemical analysis using an automated system. A potential advantage of this method is that it provides a quantitative measure that distinguishes slight differences in staining intensity and helps to eliminate inherent variability in pathologist-based scores.

In conclusion, cytoplasmic immunohistochemical expression of COX-2 and NF-κB is lower in EC than in nonmalignant endometrial lesions, and COX-2 and NF-κB have no prognostic value in EC. Our data are the first findings to compare the immunohistochemical expression of NF-κB in EC and its precursor lesions and to study its prognostic value in EC.

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