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Increased Cyclooxygenase-2 Expression in Juvenile Polyposis Syndrome

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Abstract

Background & Aims—Gastrointestinal juvenile polyps may occur in juvenile polyposis syndrome (JPS) or sporadically. JPS is an autosomal-dominant condition caused by a germline defect in *SMAD4* or *BMPR1A* in 50% to 60% of cases, and is characterized by multiple juvenile polyps, predominantly in the colorectum. JPS has an increased risk of gastrointestinal malignancy but sporadic juvenile polyps do not. Cyclooxygenase-2 (COX-2) expression is increased in gastrointestinal tumorigenesis and familial adenomatous polyposis. Inhibition of COX-2 leads to regression of colorectal adenomas in familial adenomatous polyposis patients and inhibits gastrointestinal tumorigenesis. To investigate the role of COX-2 in juvenile polyps, we compared the expression of COX-2 in juvenile polyps from a well-defined group of juvenile polyposis patients and sporadic juvenile polyps.

Methods—COX-2 expression was assessed in 24 genetically well-defined JPS patients and 26 patients with sporadic juvenile polyps using tissue microarray analysis. Two additional markers, Hu-antigen R, a stabilizer of messenger RNA, and CCAAT/enhancer-binding protein β, a transcription factor, both associated with increased COX-2 expression, also were investigated.

Results—Increased COX-2 expression in JPS patients was noted compared with patients with sporadic juvenile polyps ($P < .001$). Also, JPS patients with a *BMPR1A* germline defect had higher COX-2 expression than did JPS patients in whom no germline mutation was detected. High COX-2 levels correlated with increased cytoplasmic Hu-antigen R expression in JPS polyps ($P = .022$), but not in sporadic juvenile polyps.

Conclusions—Juvenile polyposis and sporadic juvenile polyps show distinctive expression profiles of COX-2 that may have clinical implications.

Juvenile polyps occur in about 1% of the pediatric population and most often are sporadic, solitary lesions of the colorectum. [1] These hamartomatous polyps are characterized by distorted and dilated crypts with reactive changes of the epithelium and an abundance of stroma. In

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contrast, juvenile polyposis syndrome (JPS) is an autosomal-dominant condition characterized by multiple juvenile polyps throughout the gastrointestinal tract.[2] In JPS, juvenile polyps often contain relatively less stroma, fewer dilated crypts, and more epithelial proliferative activity than their sporadic counterparts.[3] Sporadic juvenile polyps are not associated with an increased risk of gastrointestinal malignancy.[4] However, in juvenile polyposis, a recently performed person-year analysis showed a relative risk for colorectal cancer of 34% and a cumulative lifetime risk of 39%.[5]

Germline mutations in either *SMAD4* or *BMPR1A* are found in 50% to 60% of JPS cases.[6–9] The transforming growth factor-β co-receptor endoglin has been suggested as a predisposition gene for JPS, although this is still under debate.[9–11] *SMAD4*, *BMPR1A*, and endoglin are components of the transforming growth factor-β/bone morphogenetic protein signaling pathway, which is involved in the regulation of cell proliferation and differentiation.[12] Patients with a germline *SMAD4* mutation may possess a more aggressive gastrointestinal JPS phenotype with higher incidence of neoplastic change compared with those with *BMPR1A* mutation. [13–15] But much remains unknown about the molecular-genetic phenotype of juvenile polyps. The increased risk of malignancy in JPS patients and the distinctive histologic appearance of JPS polyps suggest differences in molecular biology of JPS versus sporadic juvenile polyps.

Cyclooxygenase-2 (COX-2) is a key enzyme in the conversion of arachidonic acid to prostaglandins and affects several signal transduction pathways modulating inflammation and cell proliferation.[16,17] COX-2 may play a crucial role in intestinal tumorigenesis through changes in cellular adhesion, local invasion, and inhibition of apoptosis, and is up-regulated in consecutive stages of the colorectal adenoma-carcinoma sequence in patients with sporadic colorectal cancer and in familial adenomatous polyposis.[18–20]

Hu-antigen R (HuR) and CCAAT/enhancer-binding protein β (C/EBP-β) interact with COX-2 and may be involved in regulation of COX-2 expression in juvenile polyps. HuR is an messenger RNA (mRNA)-binding protein capable of inhibiting rapid mRNA degradation and is associated with COX-2 expression. [21] Nucleocytoplasmic translocation is necessary for HuR activation.[22] C/EBP-β is a transcription factor regulating proliferation and differentiation,[23] capable of inducing COX-2 expression. [24] Increased C/EBP-β correlates with invasiveness in human colorectal cancer.[25]

In this study we compare COX-2 protein expression in polyps of a well-defined group of JPS patients with sporadic juvenile polyps using immunohistochemistry on tissue microarray. HuR and C/EBP-β expression were examined to investigate their relationship to COX-2 expression in JPS and sporadic juvenile polyps.

Methods

Tissue Selection

Eighty-two patients, diagnosed between 1985 and 2004 with one or more juvenile polyps, were identified in a retrospective search in the Department of Pathology databases of The Johns Hopkins Hospital in Baltimore, MD, and the Academic Medical Centre in Amsterdam, The Netherlands. The research was performed in accordance with the ethical guidelines of the research review committee of these institutions.

Clinical and family history data were examined and polyps were histologically re-evaluated by an experienced pathologist (G.J.A.O.) to confirm the diagnosis of JPS or sporadic juvenile polyps. Also, all JPS patients underwent thorough genetic analysis through direct sequencing and multiplex ligation-dependent probe amplification analysis.[9] JPS was defined as patients

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with 3 or more juvenile polyps and/or a well-established familial segregation and/or a germline mutation in one of the known JPS genes. Patients with sporadic juvenile polyps had a single sporadic polyp incidentally found and no family history of juvenile polyps. Sporadic juvenile polyps in patients with findings of colorectal mucosal inflammation were excluded.

A total of 50 patients (92 polyps) consisting of 24 JPS patients (median age at diagnosis, 10 y; range, 2–32 y; 65 polyps) and 26 patients with sporadic juvenile polyps (median age at diagnosis, 6 y; range, 1–61 y; 27 polyps) were selected for analysis. Of the 24 selected JPS patients, 7 (29%) had a *SMAD4* germline mutation and 6 (25%) carried a *BMPR1A* germline mutation, 2 of which had a contiguous *BMPR1A/PTEN* germline deletion.[9]

Tissue Microarray

Tissue microarrays were constructed from formalin-fixed and paraffin-embedded specimens using a custom-built instrument (Beecher Instruments, Silver Spring, MD). Three core biopsy specimens (0.6-mm cylinders) were taken from the polyp tissue and, if present, also from dysplastic foci within the polyp, in a standardized fashion, and arranged in a new recipient paraffin block. Normal mucosa was included separately when available.

Immunohistochemistry and Scoring

Immunohistochemistry for COX-2 (160112; Cayman Chemical Co., Ann Arbor, MI), HuR (19F1226), and C/EBP-β (sc-7962; Santa Cruz Biotechnology, Santa Cruz, CA) was performed as previously described.[27] Immunoreactivity of COX-2,[28] HuR,[29] and C/EBP-β[27] was quantified according to established systems as shown in Table 1. The highest score found determined the overall polyp score. Similarly, patient scores were determined by the highest polyp score found in that particular patient.

Statistical Analysis

Statistical analysis was performed using the SPSS 15.0 software package (SPSS Inc, Chicago, IL). The chi-square test, or, when appropriate, the Fisher exact test was applied to determine whether the difference in expression between groups (JPS vs sporadic) or correlation between markers within a group were statistically significant ($P < .05$). Overall patient scores were used when comparing JPS patients with patients with sporadic juvenile polyps for differences in expression of a certain marker. Correlations between markers were determined at the individual polyp level using the overall polyp score.

Results

Immunohistochemistry

A total of 50 patients (92 polyps), consisting of 24 JPS patients (65 polyps) and 26 patients with sporadic juvenile polyps (27 polyps), were analyzed. Eighty-one polyps were informative for all 3 markers. Immunohistochemical results for JPS and sporadic polyps are displayed in Figure 1. Epithelial and stromal COX-2 was assessed separately. Stromal COX-2 staining was rare, with the exception of granulation tissue, which formed a positive control. Therefore, only epithelial COX-2 data were included in our analysis. Because nuclear HuR staining was positive in all polyps it was not included in statistical analysis.

Juvenile Polyposis Versus Sporadic Juvenile Polyps

COX-2 expression was significantly higher in JPS patients compared with patients with sporadic juvenile polyps ($P < .001$) (Table 2). Of the 65 JPS polyps 14 (22%) contained dysplasia, but no dysplasia was found in sporadic juvenile polyps. To investigate a possible confounding effect of dysplasia, we determined whether dysplasia could be linked to high

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COX-2 expression. Although high COX-2 expression was relatively more common in dysplastic foci than in nondysplastic polyp tissue, this difference was not significant (P = .257). No statistically significant difference in JPS versus sporadic polyps was found in the expression of HuR (P = .292) and C/EBP-β (P = .234).

JPS patients carrying a *BMPR1A* germline mutation show a near-significant increase in COX-2 expression compared with JPS patients without germline mutation (P = .086), whereas JPS patients with a SMAD4 germline defect did not (P = .391) (Table 3).

Correlation Markers

Thirteen JPS polyps showed high expression of both COX-2 and cytoplasmic HuR. This correlation was statistically significant (P = .022). No such correlation was seen in sporadic juvenile polyps (P = .327). There was no correlation between COX-2 high phenotype and C/EBP-β positivity in either JPS polyps (P = .984), or sporadic polyps (P = .758).

Discussion

COX-2 is up-regulated in consecutive stages of the adenoma–carcinoma sequence in sporadic colorectal cancer and familial adenomatous polyposis.[18–20] Chemoprevention using selective (eg, Celecoxib Pfizer Inc, New York, NY) and nonselective (eg, Sulindac Merck & Co, Whitehouse Station, NJ) COX-2 inhibitors reduces the number and size of colorectal adenomas in these patients.[30,31] Patients with juvenile polyposis syndrome have a markedly increased relative and absolute risk for colorectal cancer.[5] In contrast, sporadic juvenile polyps are not considered to be precursors of colorectal malignancy.

We examined and compared immunostaining of COX-2 and 2 additional molecular markers involved in the regulation of COX-2 expression, C/EBP-β and HuR, in 24 JPS patients and 26 patients with sporadic juvenile polyps. We found a significantly higher COX-2 expression in JPS patients compared with those with sporadic juvenile polyps. Interestingly, although not significant, *BMPR1A* germline mutation carriers showed an increase in COX-2 expression compared with JPS patients without a detected germline mutation. These findings are in line with Kurland et al,[32] who recently described high COX-2 expression in one patient carrying a *BMPR1A* mutation. JPS patients with a *SMAD4* germline mutation on the other hand did not have increased COX-2 expression, even though *SMAD4* germline mutation carriers have been described as possessing a more aggressive intestinal phenotype.[15] The number of patients in our study group in whom a germline defect was found was limited, therefore these results need be interpreted with caution.

A subset of JPS patients had polyps with dysplastic foci but patients with sporadic juvenile polyps did not. Recently, Brazowski et al[33] showed progressively increasing COX-2 expression with increasing degree of dysplasia in JPS. Although a similar trend was seen in our JPS patients we did not find a statistical difference in COX-2 expression between dysplastic foci and nondysplastic polyp tissue. However, to rule out dysplasia as a potential confounding factor we calculated the difference in COX-2 expression in JPS versus sporadic juvenile polyps using polyp scores rather than the overall patient scores and stratified the results by dysplasia. In doing so we excluded polyps containing dysplastic foci from the analysis, that is, nondysplastic JPS polyps versus sporadic juvenile polyps. We found that COX-2 remained significantly higher in JPS compared with sporadic juvenile polyps (data not shown).

With other studies showing intestinal polyp regression through COX-2 inhibition, our results may have clinical implications for JPS patients. Future in vivo testing should be performed to determine the effect of COX-2 inhibition on gastrointestinal polyp formation in JPS animal models.[34–37] Although COX-2 inhibition has proven effective in colorectal adenoma

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prevention, the use of COX-2 inhibitors increases the risk of cardiovascular events and thus may not be suitable for routine prevention purposes.[38,39] However, the patients in these studies were above middle age (median age of patients > 50) and the findings therefore may not be applicable to children and adolescents suffering from juvenile polyposis.

HuR is an mRNA-binding protein capable of inhibiting rapid mRNA degradation by selectively binding AU-rich elements in the 3′ untranslated regions of mRNAs.[40] COX-2 mRNA contains HuR-binding AU-rich elements and cytoplasmic expression of HuR is associated with high COX-2 expression and poor prognosis in several human malignancies, including colorectal cancer.[29,41,42] Our data showed a correlation between high COX-2 expression and high cytoplasmic HuR expression in JPS, but not in sporadic juvenile polyps. However, no difference was found in cytoplasmic HuR expression in JPS versus sporadic juvenile polyps. Therefore, the difference found in correlation between COX-2 and HuR expression in JPS and sporadic juvenile polyps may be explained mainly by the difference in COX-2 expression in both groups. Also, correlation between COX-2 and HuR was found in *SMAD4*, but not in *BMPR1A* mutation carriers, whereas increased COX-2 expression was noted only in *BMPR1A* mutation carriers. HuR expression was similar in patients with a *SMAD4* or *BMPR1A* germline mutation. Based on these results it remains unclear whether HuR is involved in up-regulation of COX-2 in JPS. It is feasible that regulation of COX-2 expression is governed by different mechanisms in *SMAD4* versus *BMPR1A* mutation carriers.

C/EBP-β is a transcription factor regulating proliferation and differentiation[23] capable of inducing COX-2 expression and present in normal colorectal epithelial cells within the proliferative zone.[25] Generally, an increase in proliferative activity is seen in JPS compared with sporadic juvenile polyps. We found a C/EBP-β–positive phenotype in more than 90% of both JPS and sporadic juvenile polyps. No correlation between C/EBP-β and COX-2 expression was observed.

In summary, evaluation of COX-2 status, and COX-2–regulating molecules HuR and C/EBP-β, showed a significantly higher COX-2 expression in JPS patients compared with patients with sporadic juvenile polyps. Also, our results suggest JPS patients carrying a *BMPR1A* germline defect may have higher COX-2 expression than those in whom no germline defect was found. In this light, investigation of the effect of COX-2 inhibitors on polyp size and disease progression in JPS patients may be worthwhile. Additional research on the mechanisms of COX-2 regulation in juvenile polyps may be of interest.

Abbreviations used in this paper

C/EBP-β, CCAAT/enhancer-binding protein β; COX-2, cyclooxygenase-2; HuR, Hu-antigen R; JPS, juvenile polyposis syndrome; mRNA, messenger RNA..

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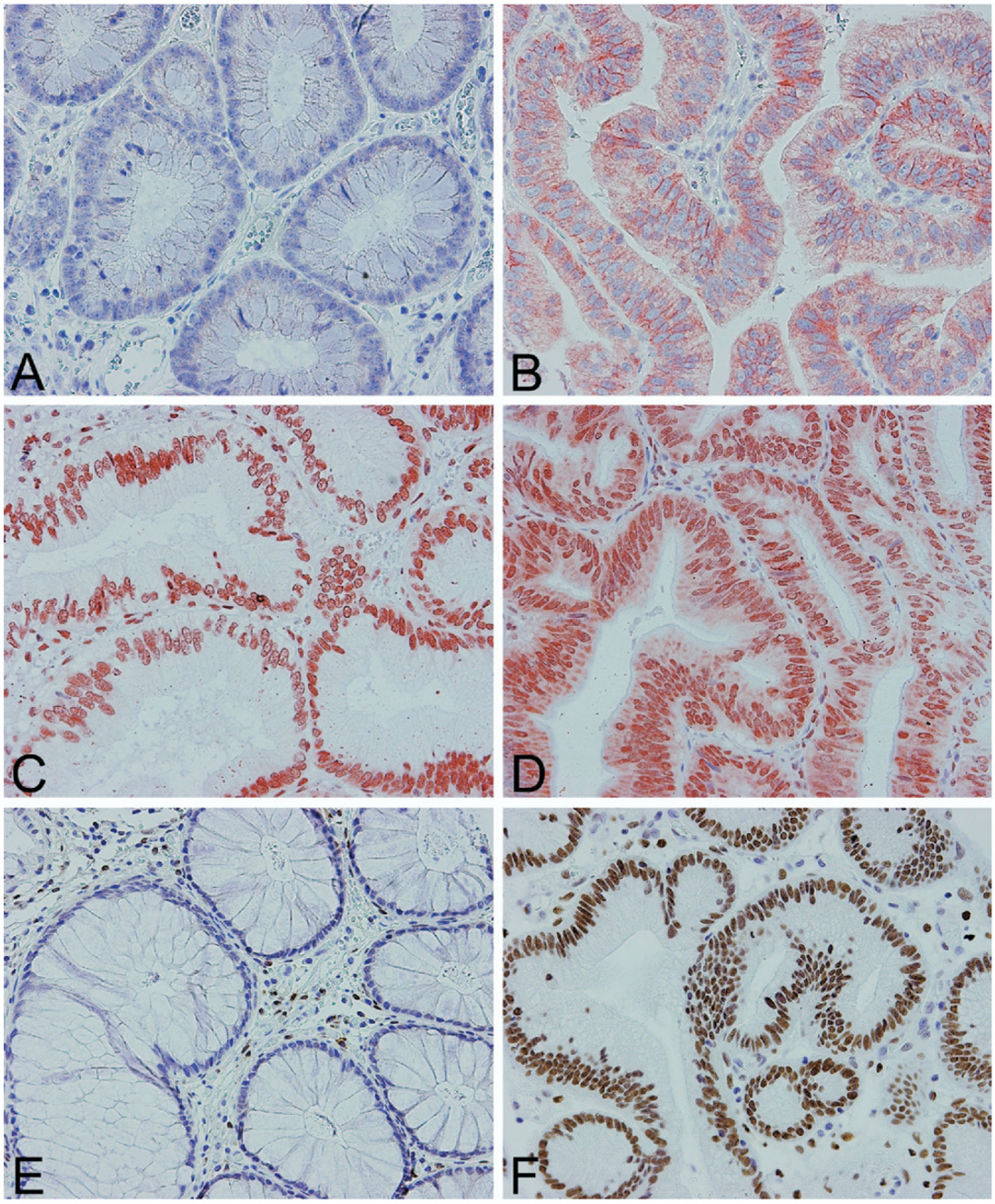

Figure 1. Immunohistochemistry on tissue microarrays for COX-2

(*A*) COX-2 low, (*B*)COX-2high, (*C*) HuR-negative cytoplasmic staining, (*D*) HuR-positive cytoplasmic staining, (*E*) C/EBP-β negative, and (*F*) C/EBP- β positive. Magnification, 20×, counterstain hematoxylin.

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Table 1

Scoring of Immunohistochemistry

COX-2	
Low	0: no staining
	1: very weak diffuse cytoplasmic staining
High	2: moderate to strong granular cytoplasmic staining in 10%–50% of cells
	3: strong intensity in >50% of cells
HuR	Nuclear and cytoplasmic staining was scored separately as positive (high) or negative (low) in epithelial cells
C/EBP-β	Nuclear staining >25% of epithelial cells

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Table 2

Immunohistochemical Results: Juvenile Polyposis Polyps Versus Sporadic Juvenile Polyps

	JPS		Sporadic juvenile polyps		
	n	**IHC**	**n**	**IHC**	**JPS vs Sporadic**
COX-2	24	54% high	24	4% high	$P < .001$
HuR	23	57% high	24	50% high	$P = .292$
C/EBP-β	22	96% positive	23	91% positive	$P = .234$

n, number of patients analyzed; IHC, immunohistochemistry.

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Table 3

COX-2 Expression in Germline Mutation Carriers Versus Nongermline Mutation Carriers

			No germline mutation	
			n	IHC
Germline mutation		**COX-2**	**11**	**36% high**
SMAD4	n	7	*P* = .391	
	IHC	57% high		
BMPR1A	n	6	*P* = .086	
	IHC	83% high		

n, number of patients analyzed; IHC, immunohistochemistry.