Expression of cyclooxygenase-2 in endometrial adenocarcinoma

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Summary

Studies have shown that COX-2 is up-regulated in several epithelial carcinomas. In this study, we wish to elucidate if endometrial cyclooxygenase-2 (COX-2) expression in endometrial adenocarcinoma is increased relative to normal endometrium. Thirty-six deparaffinized tissue sections from patients with endometrial adenocarcinoma were analyzed by immunohistochemistry for the presence of COX-2. A control group consisted of 13 age-matched patients without malignancy, who underwent surgery for uterine prolapse. Statistical analysis was performed using the Kruskal-Wallis test; differences between groups were evaluated using the Fisher's Exact Test. We found that COX-2 expression was markedly increased in 13 of 36 patients (36.1%) with endometrial adenocarcinoma: in contrast only one of 13 (7.7%) control patients demonstrated increased COX-2 expression ($p \leq 0.05$). Eight of the 13 COX-2 positive patients in the study had well differentiated adenocarcinoma; the remaining five COX-2 positive patients had moderately and poorly differentiated adenocarcinoma (4 and 1, respectively). In conclusion, COX-2 expression in the endometrium is associated with endometrial adenocarcinoma, especially of the well differentiated type. This may provide an avenue for chemoprevention of endometrial adenocarcinoma. In addition, with new selective inhibitory drugs being developed, inhibition of COX-2 may play an adjunctive role approach to standard therapy, especially for well-differentiated endometrial carcinoma. Further studies are required to investigate the role of COX-2 expression in carcinogenesis.

Key words: COX-2; Endometrial cancer; Immunohistochemistry; Aromatase.

Introduction

In the United States, endometrial cancer is the most common malignancy of the female genital tract. The American Cancer Society estimated that there were 38,300 new cases and 6,600 expected deaths from endometrial cancer in the year of 2001 [1]. In the vast majority of cases, adenocarcinoma is the predominant type (> 75%). Adenocarcinoma of the endometrium has many common histologic features with adenocarcinoma of the colon [2].

The formation of prostaglandin (PG) is initiated with metabolism of arachidonic acid by cyclooxygenases (COXs) [3]. Two isoforms of cyclooxygenase have been identified, COX-1 and COX-2. COX-1 is thought to be ubiquitous and regulates constitutive processes, whereas COX-2 is a response gene, which is expressed after stimulation by growth factors and cytokines [4]. COX-2 is also known to be a tumorogenic agent. COX-2 expression is markedly increased in carcinomas of the gastrointestinal tract [5]. In addition, accumulating clinical and epidemiological data shows that aspirin and various members of the nonsteroidal anti-inflammatory drug family inhibit growth of cancer cells [6, 11].

Based on the fact that COX-2 is tumorogenic and prostaglandin synthesis is associated with aromatase expression, we examined the expression of COX-2 in endometrial adenocarcinoma of different grades and stages to explore the association of COX-2 with endometrial adenocarcinoma.

Patients and Methods

Patient Selection

A case series of 36 patients diagnosed with endometrial adenocarcinoma between 1987 and 1998 was obtained from the files of the Department of the Pathology and Obstetrics and Gynecology of the Mount Sinai Medical Center-Chicago Medical School. Approval for this study was obtained from the hospital's Institutions Review Board. Patients had undergone radical surgery for potentially curative tumor resection. Patients were followed-up from three to 14 years. One patient died from a cancer-related cause. A control group was developed from 13 age-matched patients with no history of malignancy who had undergone surgery for uterine prolapse. The median age of the study group was 64 (mode 61.0) compared to 60.5 (mode 58.0) in the control group ($p = 0.403$). Adenocarcinomas were classified according to FIGO classification as well as the TNM classification including lymph node status after staining with hematoxylin and eosin reagent. Adenocarcinomas with other histologic types such as tumors with papillary or clear cell differentiation were excluded from the study.

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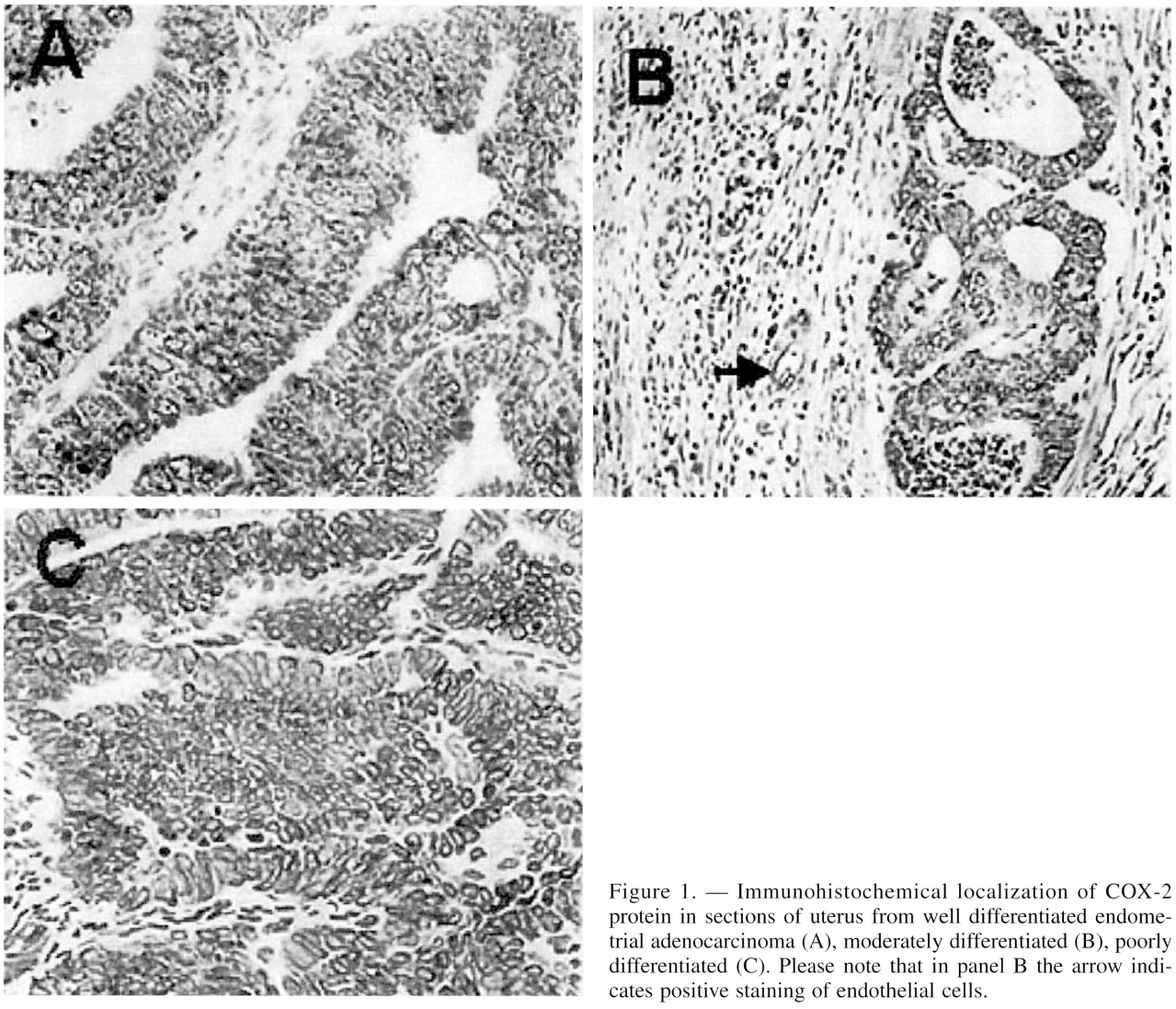

Figure 1. — Immunohistochemical localization of COX-2 protein in sections of uterus from well differentiated endometrial adenocarcinoma (A), moderately differentiated (B), poorly differentiated (C). Please note that in panel B the arrow indicates positive staining of endothelial cells.

Immunohistochemical Staining and Evaluation

Formalin-fixed and paraffin embedded adenocarcinoma specimens and control uterine specimens were cut (5mm) and deparaffinized. After blocking the endogenous peroxides with 1% hydrogen peroxide, serial sections were incubated with COX-2 antibody (Santa Cruz, CA) at a dilution of 1:200 [12]. All slides were evaluated independently by two pathologists (#1, #2), Slides were studied at magnifications of x20, x40, x100, and classified according to the percentage of the areas containing staining: 0-5%, 0; 6-35%, 1; 36-65%, 2; 66-100%, 3.

Statistical studies

Statistical analysis was performed using the Kruskal-Wallis test. Differences between groups were evaluated using the Fisher's exact test; $p \leq 0.05$ was considered to be statistically significant.

Results

COX-2 had significantly increased expression in neoplastic endometrial samples (n = 13/36, 36.1%) compared to the control group (n = 1/13, 7.7%), $p \leq 0.05$. Immunohistochemical staining for COX-2 was significantly greater in well differentiated carcinomas (n = 8/16, 50%) and moderately differentiated carcinomas (n = 4/10, 40%). Staining was only slightly increased in poorly differentiated carcinoma (n = 1/10, 10%) and in the benign control group (n = 1/13, 7.7%), p = 0.03.

The neoplastic group included 29 patients who were Stage 1A, 1B, 1C, with 11 having positive staining. Seven patients were Stage 2A or above, three of them having positive staining. No significant relationship was found between staining and the presence of lymph node metastases in six patients. Only two of these showed positive staining for COX-2. Both were Stage 3C; one was poorly-differentiated and the other moderately differentiated. In addition to the staining in endometrial epithelial cells, we also observed that vascular endothelial cells around the glands were positively stained for COX-2 (Figure 1).

Discussion

COX-1 and COX-2 catalyze the rate-limiting steps in the expression of prostaglandins and thromboxanes. COX-1 is ubiquitously expressed whereas COX-2 is induced as an immediate early gene in most cells [13, 15]. Various cytokines, tumor promoters and carcinogens induce COX-2 expression [16, 17]. Current information suggests that increased expression of COX-2 is sufficient to transform mammary epithelium into a tumorigenic state [18]. In addition, it has been shown that use of non-steroidal anti-inflammatory drugs (NSAIDs) decreases the incidence of colorectal, breast and lung cancer, which is further supported by both animal models and *in vitro* studies [19, 24].

Histologic studies have focused on adenocarcinomas of various organ systems. In our study, the significance of COX-2 expression in adenocarcinoma of the endometrium was studied. COX-2 expression was observed to be significantly increased in well-differentiated adenocarcinoma (50%). In the literature two types of endometrial adenocarcinomas have been described: Type 1 is an estrogen-related neoplasm, which tends to be of low grade. Type 2 is a more virulent form, unrelated to estrogenic stimulation [25]. In one study it was shown that carcinomas in estrogen users are more differentiated than are those in non-estrogen users [26]. In the same study it was suggested that the development of poorly differentiated adenocarcinoma is unrelated to the use of estrogen. Our findings support a relationship between estrogen effects and well-differentiated adenocarcinoma of the endometrium, with increased COX-2 expression. Eight of 16 (50%) well differentiated tumors, and four of ten (40%) moderately differentiated tumors were COX-2 positive, compared to only one of ten (10%) poorly differentiated adenocarcinomas.

The relationship of estrogen and endometrial cancer is well documented. Estrogens are synthesized from androgens in a reaction catalyzed by the cytochrome P-450 enzyme complex known as aromatase which is expressed in different tissues including endometrium. Aromatase is expressed in tumor cells and surrounding stromal cells as well. Our results revealed that vascular endothelial cells expressed COX-2 when neoplastic epithelium stained positive. In contrast, when there was no expression in tumor cells, COX-2 was absent from endothelial cells. This observation raises the possibility that COX-2 expression might be initiated by cytokines in peripheral tissue other than the end organ. Prostaglandin (PG) E_2 is known to regulate aromatase gene expression and is the product of COX-2. This is an enzyme frequently over-expressed in tumors [27]. These results suggest that PGE2, produced by COX-2, may be important in stimulating estrogen synthesis in the tumor and surrounding tissues. It is known that certain cytokines help COX-2 initiate PG expression. Bulun *et al.* have shown that through cAMP, PGs induce aromatase expression in endometrial stromal cells, leading to elevation of local estrogen synthesis (Figure 3) [28-30]. Furthermore, our results can partially be explained by the role of PG elevating the aromatase expression via COX-2 [30]. Increased secretion of PGs from inducible COX-2 present in epithelial and stromal cell compartments will result in both autocrine and paracrine actions to increase aromatase expression in tissues [31, 32]. Their results support a correlation of aromatase and COX-2 co-expression.

COX-2 elevates local estrogen effects via aromatasis. It also is an early-response gene involved in angiogenesis such as vascular endothelial growth factor and is inducible by various stimuli which are involved in proliferation, such as cyclin D1. The signaling pathways that regulate the expression of aromatase and COX-2 in neoplastic endometrium may help us identify novel targets in carcinogenesis of the endometrium. Our data imply the possibility of using COX-2 inhibitors as a chemopreventive agent, especially in well-differentiated adenocarcinoma of the endometrium. Several studies have shown recently that expression of COX-2 is significantly increased in endometrial malignancies [33-35]. Its prognostic significance needs to be further evaluated in larger prospective trials with long follow-up.

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