EXPRESSION OF CYCLOXYGENASE-2 IN HUMAN BLADDER AND RENAL CELL CARCINOMA

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INTRODUCTION

Cyclooxygenase (COX) catalyses the synthesis of prostaglandins from arachidonic acid. There are two isoforms of COX (DuBois et al., 1998; vane et al., 1998). One is constitutively expressed (COX-1) and the other is inducible (COX-2). The COX-2 gene is an immediate, early-response gene that is induced by growth factors, oncogenes, carcinogens, and tumor-promoting phorbol esters (Inoue et al., 1995; Nanayama et al., 1995; Herschman, 1996). The constitutive isoform, COX-1, is essentially unaffected by these factors.

Multiple lines of evidence have suggested that COX-2 is important in gastrointestinal carcinogenesis. COX-2 is up-regulated in various forms of gastrointestinal cancer (Eberhart et al., 1994; Ristimaki et al., 1997), whereas levels of COX-1 are relatively constant. Moreover, a null mutation for COX-2 caused a marked reduction in the number and size of intestinal polyps in APC<sup>−/−</sup> mice, a murine model of familial adenomatous polyposis (Oshima et al., 1996). In addition to the genetic evidence implicating COX-2 in tumorigenesis, newly developed selective inhibitors of COX-2 protect against gastrointestinal tumor formation (Takahashi et al., 1996; Reddy et al., 1996; Kawamori et al., 1998). Here, we investigated whether COX-2 was up-regulated in human bladder and renal cancer as well as in gastrointestinal cancer.
MATERIALS AND METHODS

Tissue samples
Tissue samples were taken from a non-necrotic area of the tumor and from adjacent non-tumorous tissue at the Department of Urology, Nippon Medical School. Informed consent was obtained from each patient. The grade of tumors was determined according to criteria adopted by WHO.

Immunohistochemistry
Tissues were fixed in formalin, embedded in paraffin, cut into 2.5 μm sections and mounted onto polylysine-coated slides. Sections were dewaxed in xylene and rehydrated in descending alcohol. They were immersed in 0.3% hydrogen peroxide to block endogenous peroxidase activity, microwaved in pH 6.0 citrate-phosphate buffer for antigen retrieval, and incubated with 10% normal goat serum to block nonspecific binding. Rabbit polyclonal antibody specific for COX-2 (IBL, Gunma, Japan) was then applied as the primary antibody, followed by a standard staining procedure using Histofine SAB-PO(R) kit (Nichirei, Tokyo, Japan). The intensity of COX-2 immunostaining was estimated on a scale from 0.5 (very low intensity) to 3 (very high intensity) in consensus of two investigators.

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

Total RNA was isolated according to the acid guanidinium thiocyanate procedure. RT-PCR was performed using COX-2 specific primers, 5'-GGTCTGGTGCTGGTCTGATGATG-3' and 5'-GTCCTTTCAGGAGAATGGTGC-3', and 5 μg total RNA as templates by Ready-To-Go RT-PCR Beads (Amersham Pharmacia Biotech). The PCR products were separated by electrophoresis on 1% agarose gel and visualized by ethidium bromide staining. For southern blot analysis, the PCR products were then transferred to a nylon membrane and hybridized with [32P]-labeled human COX-2 probe (Nanayama et al., 1995).

RESULTS AND DISCUSSION

Expression of COX-2 in Human Bladder Transitional Cell Carcinoma (TTC)

In order to examine the expression of COX-2 mRNA in human urinary bladder tissues, we first performed RT-PCR analysis. As shown in Fig. 1, COX-2 transcripts were more abundant in bladder tumors than in normal bladder tissues. In normal tissues, COX-2 transcripts were not detected.

To investigate expression and location of the COX-2 protein, immunohistochemistry was applied to bladder TCC. Positive immunoreactivity to COX-2 protein was observed in 52 of 53 TCC samples. COX-2 immunoreactivity was observed in the majority of TCC but was not detected in normal epithelium adjacent to the TCC. Interestingly, the intensity of COX-2 immunostaining was correlated with cancer grade (Fig. 2). These results suggested that an increase in COX-2 expression may be associated with the bladder carcinogenesis as well as gastrointestinal carcinogenesis, and COX-2 may be