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Immunohistochemical Expression of COX-2 in Thyroid Nodules

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Background : Recent evidence indicates that elevated COX-2 expression is associated with the carcinogenesis of numerous neoplasms. In this study, we investigated COX-2 expression in various thyroid specimens in order to elucidate its physiological role in pathologic conditions, and to evaluate the efficiency of COX-2 protein expression as a molecular marker of malignancy in the thyroid gland.

Methods : COX-2 expression was studied immunohistochemically in 19 papillary carcinomas, 8 follicular carcinomas, 14 follicular adenomas, 2 Hürthle cell carcinomas, 4 Hürthle cell adenomas, 8 nodular hyperplasias, 3 Graves' diseases, 3 Hashimoto's thyroiditis, 2 medullary carcinomas, 1 anaplastic carcinoma, and 20 normal thyroid tissues.

Results : COX-2 staining was not seen in any of the normal thyroid, Graves' disease, or nodular hyperplasia specimens. In contrast, COX-2 staining was observed in all of papillary carcinomas, Hashimoto's thyroiditis, Hürthle cell carcinomas, and Hürthle cell adenomas tissues. Moreover, 7 of 8 follicular carcinomas and 11 of 14 follicular adenomas showed COX-2 staining.

Conclusion : These results indicate that COX-2 is not useful as a marker of malignancy. Since COX-2 expression was evident in follicular adenomas and in papillary and follicular carcinomas. Thus, the enzyme may be involved in the early process of thyroid tumorigenesis.

Key Words : COX-2, Thyroid nodule, Immunohistochemistry

INTRODUCTION

Thyroid nodule is a common disorder whose prevalence on clinical evaluation varies from 1~7% according to iodine intake. However, thyroid cancer represents just 1~2% of all malignancies, and only 5~24% of thyroid nodules treated surgically are malignant. Fine-needle aspiration biopsy (FNAB) is the first line tool for the evaluation of thyroid nodules. However, misdiagnoses may occur, due to either an insufficiency or the unsuitability of the aspirated material for cytologic evaluation or to a sampling mistake. Furthermore, the main limitation of FNAB is its lack of sensitivity in the evaluation of follicular neoplasms, due to its inability to differentiate follicular adenoma from follicular carcinoma. If a more reliable marker for the presence of thyroid cancer were available for preoperative evaluation, unnecessary thyroid surgeries could be avoided.

Cyclooxygenase (COX) catalyzes the formation of prostaglandins from arachidonic acid[1]. Recent animal studies suggest that prostaglandins play a key role in tumor progression via their influence on angiogenesis, tumor growth, and metastasis[2-4]. At least two COX enzymes are present in humans: COX-1 and COX-2. COX-1 is known as a housekeeping gene and is constitutively expressed in most tissues. In contrast, COX-2 is an early response gene and is induced by various factors, such as growth factors, oncogenes, cytokines, and carcinogens. Moreover, COX-2 is up-regulated in numerous neoplasms, including colorectal[5], pancreatic[6], prostate[7], esophageal[8], lung[9], gastric[10], and skin carcinoma[11].

Recently, Specht et al.[12] reported that the COX-2 mRNA

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level is higher in malignant thyroid nodules than in adjacent normal tissue or in benign thyroid nodules. Thus, we investigated COX-2 expression in various thyroid specimens in order to elucidate its physiological role in pathologic conditions, and to evaluate the efficiency of COX-2 protein expression as a molecular marker of malignancy in the thyroid gland, and thus its potential usefulness in the differentiation of benign and malignant tumors.

MATERIALS AND METHODS

1) Specimens

Sixty-four paraffin-embedded thyroid specimens were obtained from surgical resections performed at Cheonan Hospital, Soon chun hyang University, Cheonan, Korea. These consisted of 19 papillary carcinomas, 8 follicular carcinomas, 14 follicular adenomas, 2 Hürthle cell carcinomas, 4 Hürthle cell adenomas, 8 nodular hyperplasias, 3 Graves' diseases, 3 Hashimoto's thyroiditis, 2 medullary carcinomas, 1 anaplastic carcinoma, and 20 normal thyroid tissues. Normal thyroid tissue samples were taken from histologically normal areas adjacent to neoplastic lesions.

2) Immunohistochemistry

Immunohistochemical analysis of COX-2 was performed using established protocols. Briefly, paraffin-embedded tissue was cut into 5 m section and dried for 1h at 57℃ in an oven. After routine deparaffination and rehydration, tissue sections were microwaved for 20 min in 0.01 M sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 30 min, and sections were then incubated with rabbit polyclonal antibody against human COX-2 (Cayman Chemical, Ann Arbor, MI) at a dilution of 1:10 for 60 min at room temperature. All slides were then washed 3 times for 3 min each with phosphate-buffered saline (PBS). Samples were incubated with PicTure-plus bulk kit (Zymed Lab, San Francisco, CA), i.e., Zymed's HRP polymer detection system, for 20 min at room temperature, washed and incubated with Liquid DAB substrate kit (Zymed Lab, San Francisco, CA) for 5 min, and then counterstained with Meyer's hematoxylin for 5 min and mounted. For negative controls, incubation with the primary antibody was omitted. Staining was scored as follows: (-) = absent, (+/-) = weak or focal staining, and (+) = moderate to strong staining in all or most epithelial cells.

Table 1. Expression of COX-2 in thyroid lesions

Diagnosis (No of cases)	+	+/-	-
Papillary carcinoma (19)	19	0	0
Follicular carcinoma (8)	6	1	1
Hürthle cell carcinoma (2)	1	1	0
Medullary carcinoma (2)	1	1	0
Anaplastic carcinoma (1)	0	1	0
Follicular adenoma (14)	10	1	3
Hürthle cell adenoma (4)	3	1	0
Nodular hyperplasia (8)	0	0	8
Hashimoto's thyroiditis (3)	3	0	0
Graves' disease (3)	0	0	3
Normal tissue (20)	0	0	20

+: strong to moderate staining of all or most of epithelial cells
+/-: weak or focal staining of epithelial cells
-: no staining of epithelial cells.

RESULTS

Table 1 summarizes the results obtained for COX-2 immunostaining in the thyroid tissues of the 64 patients. COX-2, when expressed, was found in the cytoplasm of follicular cells. COX-2 staining was not seen in any of the 20 normal thyroid (Figure 1A), 3 Graves' disease, or 8 nodular hyperplasia (Figure 1B) specimens. In contrast, COX-2 staining was observed in all of 19 papillary carcinomas (Figure 1C), 3 Hashimoto's thyroiditis (Figure 1D), 2 Hürthle cell carcinomas, and 4 Hürthle cell adenomas, and in 7 of 8 follicular carcinomas (Figure 1E) and 11 of 14 follicular adenomas (Figure 1F). One of 2 medullary carcinomas showed diffuse COX-2 staining, but the another showed focal staining. One anaplastic carcinoma showed weak COX-2 positivity.

DISCUSSION

In contrast to the findings of Smith et al[13], we did not observe COX-2 expression in the normal thyroid epithelium. This result was in accordance with reports by Cornetta et al.[14] and Specht et al.[12] Moreover, in this study, we found increased COX-2 expression in Hashimoto's thyroiditis, follicular adenoma and in well-differentiated thyroid cancer.

Chronic inflammation may enhance carcinogenesis and aid neoplastic growth and progression by promoting genomic instability[15]. For example, ulcerative colitis has been shown to carry an increased risk of colon cancer development[16]. In addition, patients with Hashimoto's thyroiditis have a 10 to 40% increased risk of developing papillary thyroid carcinoma[17, 18]. The association of lymphocytic infiltrates with papillary thyroid carcinomas and Hashimoto's thyroiditis suggest that inflammatory conditions within the thyroid may promote the development of thyroid cancer.

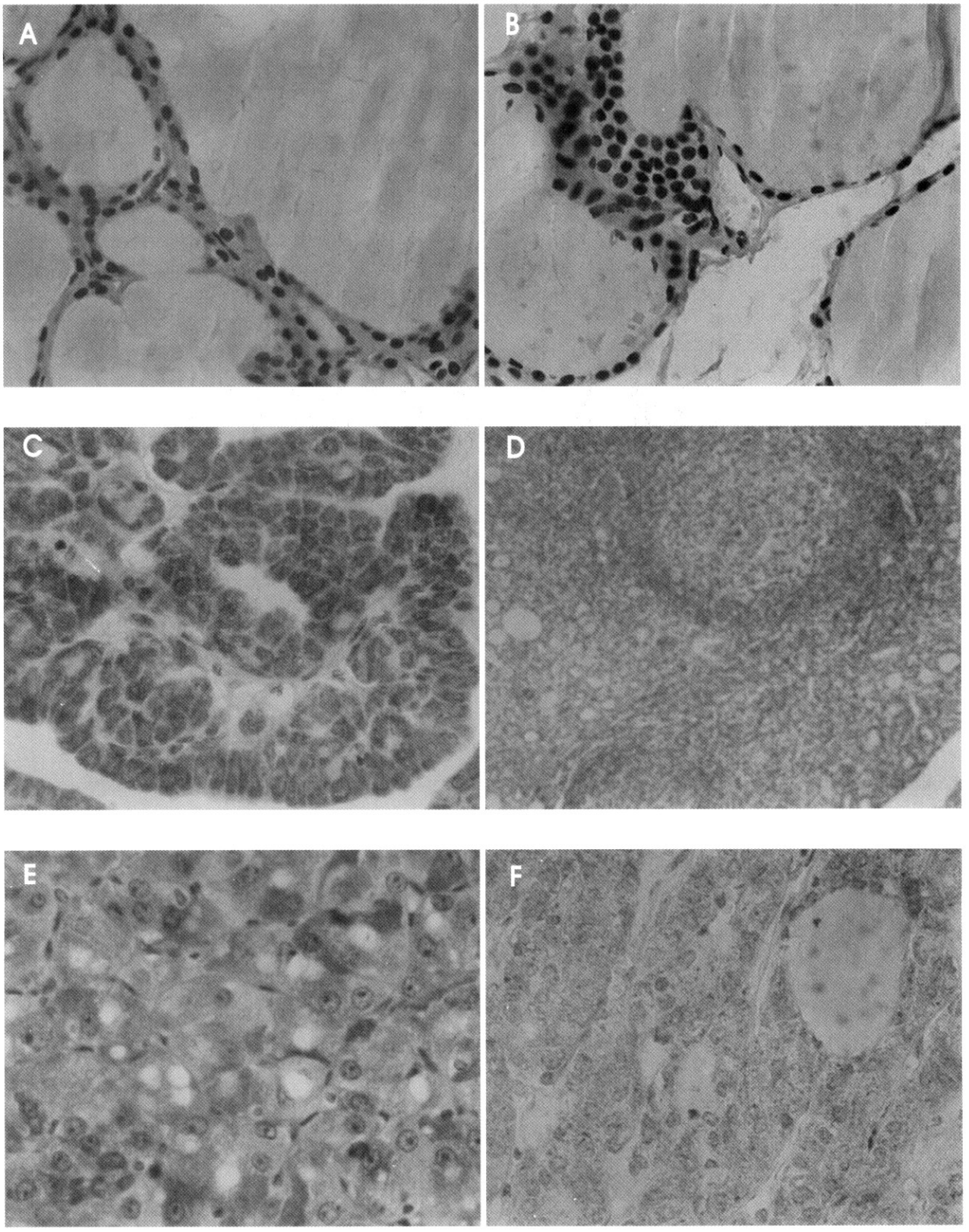

Figure 1. Immunohistochemical analysis of COX-2 in thyroid tissues. A: normal tissue (×400), B: adenomatous goiter (×400), C: papillary carcinoma (×400), D: Hashimoto's thyroiditis (×100), E: follicular carcinoma (×400), F: follicular adenoma (×400).

The incidence of COX-2 over-expression in follicular carcinoma did not significantly differ from that of follicular adenoma, which indicates that COX-2 is not useful as a marker of malignancy. Since COX-2 expression was evident in follicular adenomas, and papillary and follicular carcinomas, this enzyme may be involved in the early processes of thyroid tumorigenesis.

The cyclooxygenase pathway has been implicated as a mediator of inflammation and cellular growth in the thyroid. Berg et al.[19] demonstrated that IL-1 and TNF, which are two well-known proinflammatory mediators of thyroiditis, induced COX-2 expression in thyroid epithelial cells. Based on immunohistochemistry, immunoblot, and Northern analysis results, Smith et al.[13] reported that COX-2 is constitutively expressed in multinodular goiter, Graves' disease and in papillary carcinoma. In contrast, Specht et al.[12] reported that the levels of COX-2 mRNA and protein are elevated in human thyroid cancer as compared with adjacent normal tissue. In particular, they found no expression of COX-2 by immunohistochemistry, in contrast to Smith's result. Nose et al.[20] reported that stepwise increments in the over-expression of COX-2 were shown by epithelial cells of Hashimoto's thyroiditis, follicular adenoma, papillary carcinoma, and follicular carcinoma. Cornetta et al.[14] also reported that COX-2 expression was observed in Hashimoto's thyroiditis, and in papillary and follicular carcinoma, but not in normal thyroid tissue, multinodular goiter, or in anaplastic carcinoma. Recently Ito et al.[21] reported that positive COX-2 staining was only observed occasionally in normal follicles or stromal cells, and that COX-2 over-expression was found in only 20% of follicular adenomas, 41% of follicular carcinomas, and in 81% of papillary carcinomas.

In our study, COX-2 was expressed in all 19 papillary carcinomas, 7 of 8 follicular carcinomas, and 11 of 14 follicular adenomas, which is a higher rate of positivity expression than found by Ito et al[21]. But, Cornetta et al.[14] reported that 4 of 5 papillary carcinomas and both 2 follicular carcinomas were COX-2 positive, which is similar to our result.

Prostaglandins, the products of COX-2 activity, have been implicated in carcinogenesis via multiple mechanisms, such as by promoting angiogenesis[22], inhibiting apoptosis[23], increasing malignant cell invasion[24], stimulating cell proliferation[25], and inhibiting immune surveillance[2].

Moreover, there is evidence that nonsteroidal anti-inflammatory (NSAIDs) can lower the risk of developing certain epithelial cancers and inhibit carcinogenesis. Epidemiological studies have demonstrated a 50% reduction in the rate of mortality from colorectal cancer in patients taking NSAIDs[26], and regular NSAIDs use can significantly reduce the risk of developing breast cancer by approximately 50%[27]. In addition, the risk of certain esophageal and gastric cancers is significantly reduced by the regular administration of NSAIDs[28].

No epidemiologic study has found that NSAIDs or selective COX-2 inhibitors can reduce thyroid tumor development or are effective in the treatment of thyroid tumor. But, based on our findings of an increased expression of COX-2 in thyroid tumor, we expect that use of COX-2 may be effective in the prevention or treatment of thyroid nodules. Thus further study should be conducted to determine whether NSAIDs or selective COX-2 inhibitors lower the risk of developing thyroid nodule and/or the development of thyroid cancer.

REFERENCES

1) Vane JR, Bakhle YS, Botting RM. *Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 39:97-120, 1998*
2) Huang M, Stolina M, Sharma S, Mao JT, Zhu L, Miller PW, Wollman J, Herschman H, Dubinett SM. *Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. Cancer Res 58:1208-1216, 1998*
3) Eli Y, Przedecki F, Levin G, Kariv N, Raz A. *Comparative effects of indomethacin on cell proliferation and cell cycle progression in tumor cells grown in vitro and in vivo. Biochem Pharmacol 61:565-571, 2001*
4) Sawaoka H, Tsujii S, Tsujii M, Gunawan ES, Sasaki Y, Kawano S, Hori M. *Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. Lab Invest 79:1469-1477, 1999*
5) Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. *Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 107:1183-1188, 1994*
6) Tucker ON, Dannenberg AJ, Yang EK, Zhang F, Teng L, Daly JM, Soslow RA, Masferrer JL, Woemer BM, Koki AT, Fahey TJ. *Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res 59:987-990, 1999*
7) Yoshimura R, Sano H, Masuda C, Kawamura M, Tsubouchi Y, Chargui J, Yoshimura N, Hla T, Wada S. *Expression of cyclooxygenase-2 in prostate carcinoma. Cancer 89:589-596, 2000*
8) Shamma A, Yamamoto H, Doki Y, Okami J, Kondo M, Fujiwara Y, Yano M, Inoue M, Matsuura N, Shiozaki H, Monden M. *Up-regulation of cyclooxygenase-2 in squamous carcinogenesis of the esophagus. Clin Cancer Res 6:1229-1238, 2000*
9) Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki K, Nakamura S, Ogawa M, Mitsudomi T, Sugiura T, Takahashi T. *Increased expressed of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 58: 3761-*3764, 1998
10) Ristamaki A, Honkanen N, Jankala H, Sipponen P, Harkonen M. *Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 57:1276-1280, 1997*
11) Higashi Y, Kanekura T, Kanzaki T. *Enhanced expression of*

cyclooxygenase (COX)-2 in human skin epidermal cancer cells: evidence for growth suppression by inhibiting Cox-2 expression. Int J Cancer 86:667-671, 2000

12) Specht MC, Tucker ON, Hocever M, Gonzalez D, Teng L, Fahey III TJ. *Cyclooxygenase-2 expression in thyroid nodules. J Clin Endocrinol Metab 87:358-363, 2002*

13) Smith TJ, Jennings TA, Sciaky D, Cao HJ. *Prostaglandin-endoperoxide H synthase-2 expression in human thyroid epithelium. J Biol Chem 274:15622-15632, 1999*

14) Cornetta AJ, Russell JP, Cunnane M, Keane WM, Rothstein JL. *Cyclooxygenase-2 expression in human thyroid carcinoma and Hashimoto's thyroidtis. Laryngoscope 112:238-242, 2002*

15) Prescott SM, Fitzpatrick FA. *Cyclooxygenase-2 and carcinogenesis. Biochim Biophys Acta 1470:M69-M78, 2000*

16) Morson BC. *Precancer and cancer in inflammatory bowel disease. Pathology 17:173-180, 1985*

17) Ott RA, McCall AR, McHenry C. *The incidence of thyroid carcinoma in Hashimoto's thyroiditis. Am Surg 53:442-445, 1987*

18) Okayasu I, Fujiwara M, Hara Y, Tanaka Y, Rose NR. *Association of chronic lymphocytic thyroiditis and thyroid papillary carcinoma. A study of surgical cases among Japanese, and white and African Americans. Cancer 76:2312-2318, 1995*

19) Berg J, Stocher M, Bogner S, Wolfl S, Pichler R, Stekel H. *Inducible cyclooxygenase-2 gene expression in the human thyroid cell line Nthy-ori3-I. Inflamm Res 49:139-143, 2000*

20) Nose F, Ichikawa T, Fujiwara M, Okayasu I. *Up-regulation of cyclooxygenase-2 expression in lymphocytic thyroiditis and thyroid tumors. Am J Clin Pathol 117:546-551, 2002*

21) Ito Y, Yoshida H, Nakano K, Takamura Y, Miya A, Kobayashi K, Yokozawa T, Matsuzuka F, Matsuura N, Kuma K, Miyauchi A. *Cyclooxygenase-2 expression in thyroid neoplasms. Histopathol 42:492-497, 2003*

22) Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. *Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 93:705-716, 1998*

23) Tsujii M, DuBois RN. *Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 83:493-501, 1995*

24) Tsujii M, Kawano S, DuBois RN. *Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 94:3336-3340, 1997*

25) Sheng J, Shao J, Morrow JD, Beauchamp RD, DuBois RN. *Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 58:362-366, 1998*

26) Thun MJ, Namboodiri MM, Heath CW. *Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 325:1593-1596, 1991*

27) Schapira DV, Theodossiou C, Lyman GH. *The effects of NSAIDs on breast cancer prognostic factors. Oncology Reports 6:433-435, 1999*

28) Farrow DC, Vaughan TL, Hansten PD. *Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal* and *gastric cancer. Cancer Epidemiol Biomarkers Prev 7:97-102, 1998*