

## Immunohistochemical Demonstration of Membrane-bound Prostaglandin E<sub>2</sub> Synthase-1 in Papillary Thyroid Carcinoma

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Microsomal prostaglandin E<sub>2</sub> synthase-1 (mPGES-1) is an inducible enzyme that catalyzes the conversion of prostaglandin (PG) H<sub>2</sub> to PGE<sub>2</sub> in downstream of cyclooxygenase-2 (COX-2). Recent studies have obtained *in vitro* evidence that PGE<sub>2</sub> participates in carcinogenesis, angiogenesis, and induction of matrix metalloproteinase-9 (MMP-9), which plays a crucial role in cancer invasion. However, implications for mPGES-1 in thyroid carcinomas remain to be determined. To address this issue, we performed an immunohistochemical analysis for mPGES-1, COX-2 and MMP-9 in 20 papillary thyroid carcinoma (PTC) patients. mPGES-1 immunoreactivity was localized in the cytoplasm of carcinoma cells in 19 cases, with an intensity that tended to be distinct at the interface between the tumor and the surrounding non-neoplastic tissue. Staining was more intense in regions with papillary arrangement, while it was less intense in regions with trabecular or solid arrangement. In many cases, immunohistochemical localization of COX-2 and MMP-9 resemble that of mPGES-1. Taken together, our results suggest the involvement of mPGES-1 in proliferation and differentiation of PTC as well as local invasion of PTC.

**Key words:** cyclooxygenase-2, immunohistochemistry, matrix metalloproteinase, papillary thyroid carcinoma, prostaglandin E synthase

### I. Introduction

Recent studies have obtained *in vitro* evidence that prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) participates in carcinogenesis [1, 14, 17, 20] and angiogenesis [16, 18]. The biosynthesis of PGE<sub>2</sub> requires three sequential enzymic reactions: the release of arachidonic acid from membrane glycerophospholipids by phospholipase A<sub>2</sub>, the conversion of arachidonic acid to the unstable intermediate PGH<sub>2</sub> by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2), and the isomerization of PGH<sub>2</sub> to PGE<sub>2</sub> by PGE<sub>2</sub> synthase (PGES) [EC 5.3.99.3] (Fig. 1). COX-2 and PGES collaboratively mediate the induction of matrix metalloproteinase-9 (MMP-9)

[2], which plays a crucial role in cancer invasion by basement membrane degradation [8].

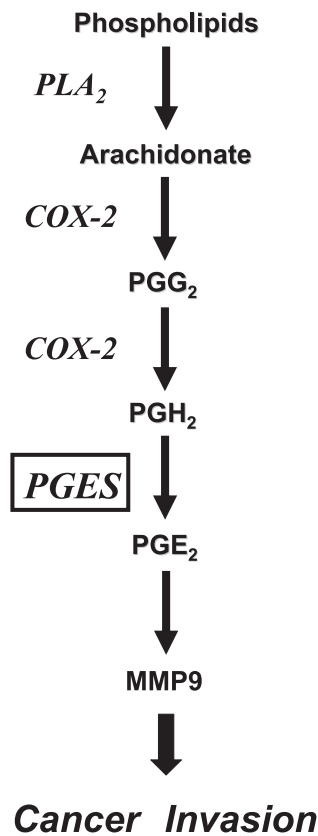
It has been shown that COX-2 is involved in the pathomechanisms of thyroid carcinomas and chronic thyroiditis (CT) [4, 11]. However, implications for PGES in thyroid carcinomas remain to be determined. To address this issue, we performed an immunohistochemical analysis for membrane-bound PGES-1 (mPGES-1), a well characterized isoform of PGES, as well as COX-2 and MMP-9, in surgically resected thyroid gland tissues including papillary thyroid carcinoma (PTC).

### II. Materials and Methods

#### *Subjects and tissue preparation*

This investigation was carried out on archival, formalin (20%)-fixed, paraffin-embedded materials of 20 sporadic PTC patients who underwent thyroidectomy, at Tokyo

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**Fig. 1.** Schematic diagram of the COX-2/PGES-1 catalyzed PGE<sub>2</sub> biosynthesis pathway in carcinoma invasion. COX-2, cyclooxygenase-2; MMP-9, matrix metalloproteinase-9; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PGES, membrane-bound prostaglandin E<sub>2</sub> synthase.

Women's Medical University Hospital. This study was performed after obtaining written informed consent from the patients examined.

#### Primary antibodies

The primary antibodies employed in immunohistochemistry were rabbit polyclonal IgG against mPGES-1 (Cayman Chemical, Ann Arbor, MI, USA; diluted 1:300), rabbit polyclonal IgG against COX-2 (Cayman Chemical; diluted 1:300), and mouse monoclonal IgG against MMP-9 (Daiichi Fine Chemical, Toyama, Japan; diluted 1:500).

#### Immunohistochemical analysis

Multiple 3- $\mu$ m-thick sections of each material were used for hematoxylin-eosin staining and immunohistochemical staining. For the latter staining, sections were deparaffinized, rehydrated, quenched for 5 min at room temperature with 3% H<sub>2</sub>O<sub>2</sub>, rinsed in phosphate-buffered saline (PBS), pH 7.6, processed with microwaving (95°C, 400 W, 20 min) in 10 mM citrate buffer, pH 6.0 for mPGES-1 and MMP-9 staining and 1 mM ethylenediamine N,N,N',N'-tetraacetic acid, pH 8.0 for COX-2, pretreated for 30 min at room temperature with 3% nonimmune animal serum in PBS, and

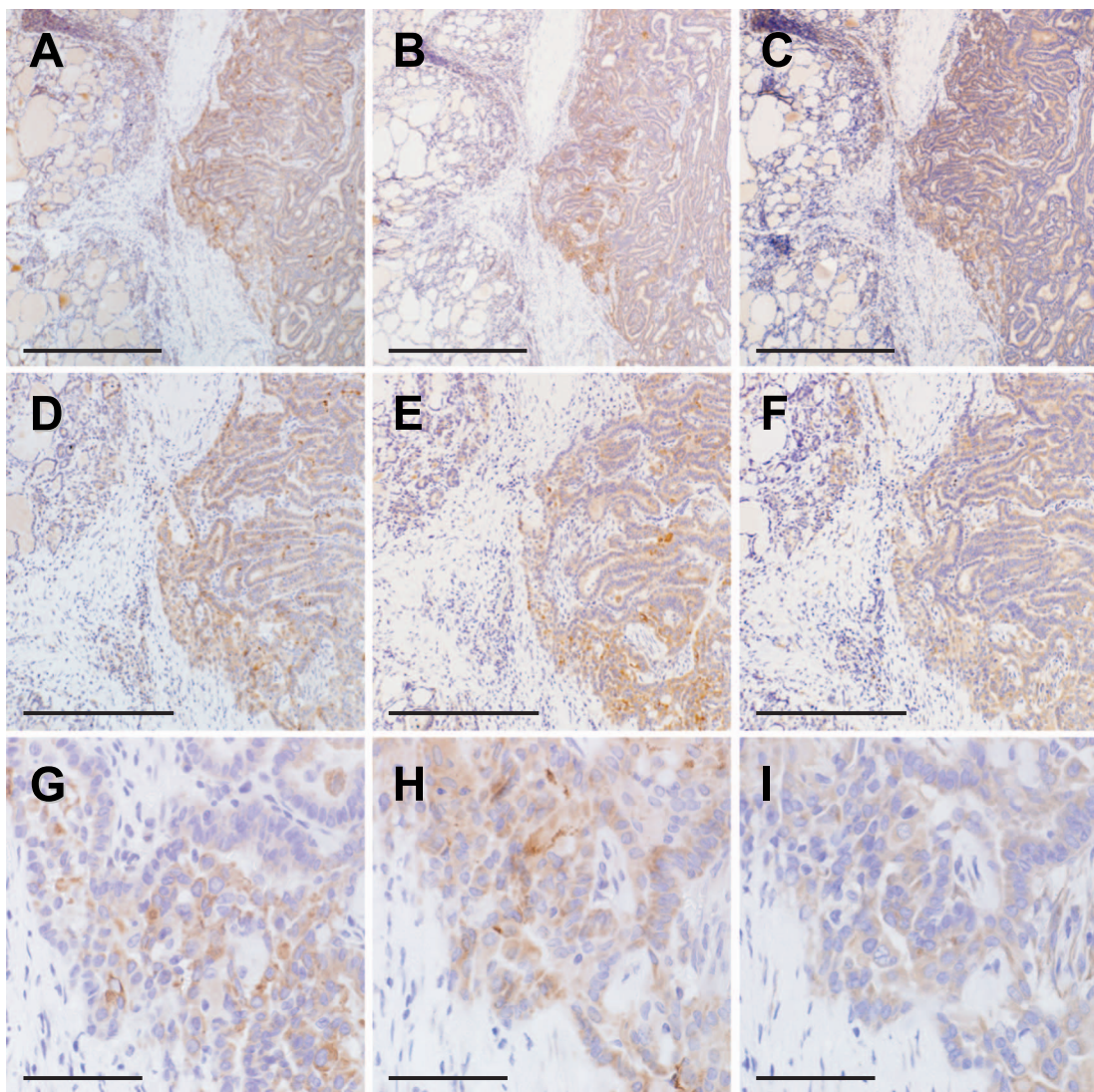
then incubated overnight at 4°C with the primary antibodies. Antibody binding was visualized by the avidin-biotin-immunoperoxidase complex method using the appropriate Vectastain ABC kits (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions, with 3,3'-diaminobenzidine tetrahydrochloride and hematoxylin as the chromogen and the counterstain, respectively. Sections from which the primary antibodies were omitted or sections which were incubated with nonimmune serum derived from the same animal species as those producing the antibodies served as negative reaction controls.

### III. Results

Of the 20 PTC cases, 19 showed focal strong mPGES-1 immunoreactivity, while the other one showed diffuse weak immunoreactivity. The immunoreactivity was localized in the cytoplasm of carcinoma cells in all of the PTC cases, and was prominent at the interface between the tumor and the surrounding non-neoplastic parenchyma (Fig. 2B, E, H). Staining was more intense in regions displaying stromal invasion with papillary arrangement (Fig. 3A), and by contrast it was less intense in regions displaying trabecular arrangement (Fig. 3B) and solid nest formation (Fig. 3C). In many cases, immunohistochemical localization of COX-2 (Fig. 2A, D, G) and MMP-9 (Fig. 2C, F, I) resembled that of mPGES-1. However, immunoreactivities for COX-2 and MMP-9 were diffuse and uniform in PTC cells of 13 cases and four cases, respectively; of the latter four cases, the mPGES-1 immunoreactivity was weak in one case and strong in three cases. Of the 20 PTC cases, seven were with CT, and the rest were not associated with any other thyroid disease. In the seven CT cases, non-neoplastic thyroid parenchyma demonstrated scattered formation of lymph follicles and swelling of follicular epithelial cells that often had eosinophilic cytoplasm and showed papillary arrangement. Immunoreactivities for mPGES-1 and MMP-9 were detected in germinal center lymphocytes and papillary-arranged follicular epithelial cells in five cases (Fig. 4A–C), but were undetectable in intact follicular epithelial cells in all of the CT cases. Immunohistochemical localization of COX-2 was similar to that of mPGES-1 and MMP-9 in three CT cases (Fig. 4A–C). Normal thyroid parenchyma did not show any marked staining for mPGES-1, COX-2 or MMP-9 (Fig. 4A–C).

### IV. Discussion

To date, three isoforms of PGES have been identified in mammals: mPGES-1, mPGES-2 and cytosolic PGES (cPGES) [7, 9]. mPGES-1 is localized in the microsomal membrane, induced by proinflammatory stimuli, and acts in concert with COX-2 to maintain and amplify inflammatory activity. mPGES-2 is localized in the Golgi apparatus membrane, constitutively expressed, and functionally coupled with COX-1 and COX-2. cPGES is localized in the cytosol, constitutively expressed, and functionally linked to COX-1.

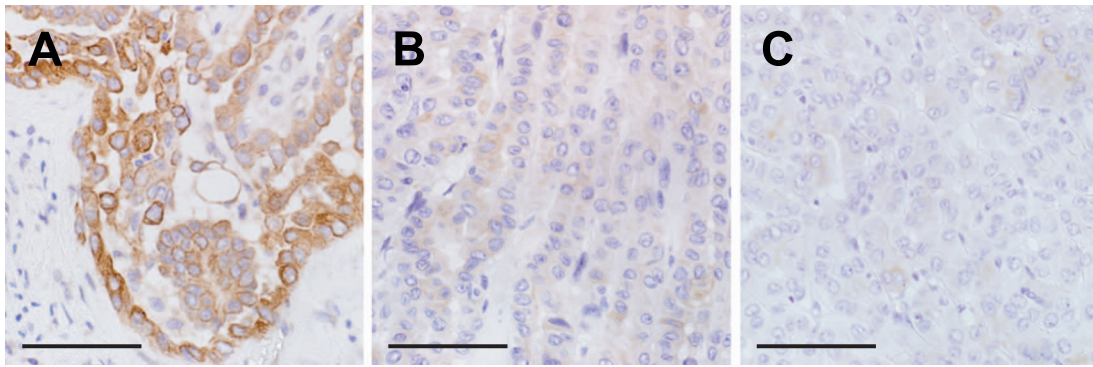


**Fig. 2.** Photomicrographs of PTC tissue sections immunostained for COX-2 (A, D, G), mPGES-1 (B, E, H) and MMP-9 (C, F, I). Series of panels (A–C), (D–F) and (G–I) indicate the same regions including the interface between PTC tissue (each right half) and non-neoplastic thyroid parenchyma (each left half) on consecutive sections, respectively. Bars=1 mm (A–C), 500  $\mu$ m (D–F), 100  $\mu$ m (G–I). ABC method using DAB. ABC, avidin-biotin-immunoperoxidase complex; DAB, 3,3'-diaminobenzidine tetrahydrochloride; PTC, papillary thyroid carcinoma.

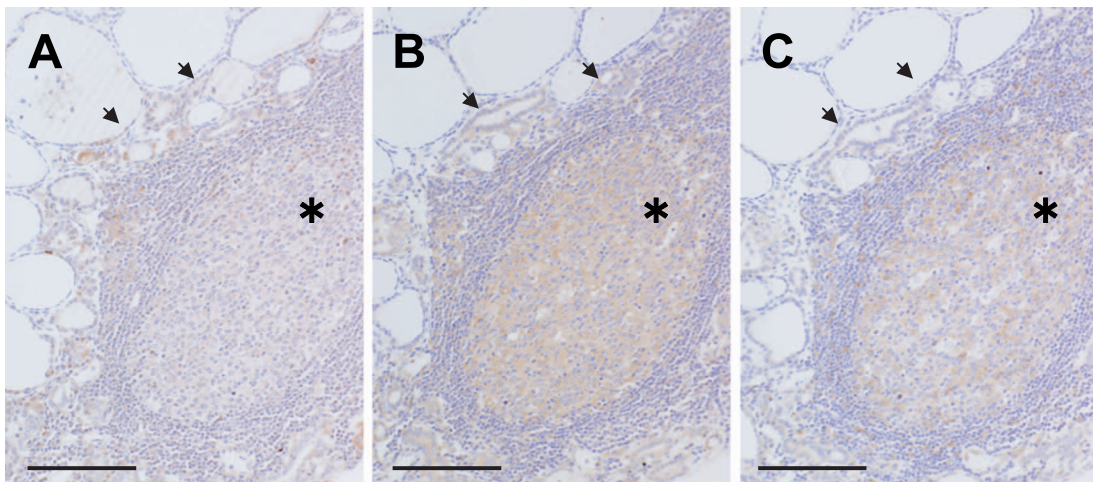
Thus, as our results indicated, it is conceivable that the inducible isoform mPGES-1 immunoreactivity was focally prominent in PTC lesions, suggesting that the levels of mPGES-1 induction stimuli vary in the sites of the lesions. Previous studies have indicated the overexpression of mPGES-1 in cancers of the lung [21], colon [22], stomach [19], head and neck [3], endometrium [6], and penis [5]. However, there is no precedent showing immunohistochemical detection of PGES in thyroid tissues, and this is the first report showing mPGES-1 expression in PTC. Several studies have documented the pathological actions of PGE<sub>2</sub> as a PGES product. PGE<sub>2</sub> activates epidermal growth factor receptor [14], peroxisome proliferator-activated receptor  $\delta$  [20], and Wnt signaling [1] in carcinogenesis. PGE<sub>2</sub> also induces expression of vascular endothelial growth factor,

basic fibroblast growth factor, and MMP-9 in carcinoma cells and stromal cells through PGE receptor EP2 that promotes cancer angiogenesis [16, 18]. Thus, the COX-2/mPGES-1-catalyzed PGE<sub>2</sub> production cascade has been of great interest to oncologists as a possible therapeutic target.

It is known that CT increases risk of the pathogenesis of PTC in a proportion varying from 10 to 40% [12, 13]. A recent study demonstrated the upregulation of COX-2 in both PTC and CT lesions, suggesting the involvement of inflammatory processes in carcinogenesis [4, 11]. In relation to this, it is noteworthy that our study showed that both COX-2 and mPGES-1 were restricted to these lesions but undetectable in morphologically intact thyroid parenchyma. Our finding of the colocalization of mPGES-1 with MMP-9 at the interface between the tumor and the surrounding non-



**Fig. 3.** Photomicrographs of PTC lesions immunostained for PGES-1. Panels (A), (B) and (C) indicate foci of papillary arrangement, trabecular arrangement, and solid nest formation of PTC, respectively. Panel (A) is from the interface of stromal invasion, and panels (B) and (C) are from the central core of a tumor. Bars=100  $\mu$ m. ABC method using DAB. ABC, avidin-biotin-immunoperoxidase complex; DAB, 3,3'-diaminobenzidine tetrahydrochloride; PTC, papillary thyroid carcinoma.



**Fig. 4.** Photomicrographs of CT lesions immunostained for COX-2 (A), mPGES-1 (B) and MMP-9 (C). Immunoreactivities for COX-2 (A), mPGES-1 (B) and MMP-9 (C) were detected in germinal center lymphocytes (asterisks) and papillary-arranged follicular epithelial cells (arrows) but undetectable in intact follicular epithelial cells (each left upper side). Bars=200  $\mu$ m. ABC method using DAB. ABC, avidin-biotin-immunoperoxidase complex; CT, chronic thyroiditis; DAB, 3,3'-diaminobenzidine tetrahydrochloride; PTC, papillary thyroid carcinoma.

neoplastic tissue suggests a close link between these two enzymes. This supports *in vitro* evidence that the mPGES-1 product PGE<sub>2</sub> induces MMP-9 [2], which contributes to carcinoma invasion [8].

Another intriguing finding that mPGES-1 expression levels were greater in PTC cells displaying papillary arrangement compared to those showing trabecular or solid arrangement points to the possibility that mPGES-1 up-regulation may be involved in the differentiation of PTC; papillary arrangement may indicate the state of well differentiation of carcinoma, while trabecular or solid arrangement may indicate the state of poor differentiation. It has been shown that RET/PTC rearrangement is detectable in approximately 30% of the adult sporadic PTC cases [10]. Intracellular tyrosine kinase domain of RET is coupled with an N-terminal fragment of various unrelated genes,

and RET/PTC rearrangement occurs in the early step of carcinogenesis of PTC. The two most common rearrangement types, RET/PTC1 and RET/PTC3, upregulate COX-2 and mPGES-1 expression levels in cultured thyroid cells [15]. These observations suggest that mPGES-1 expression triggers PTC carcinogenesis. Finally, it remains to be determined whether mPGES-1 expression levels may have relevance to the prognosis of PTC via direct invasion or hematogenous or lymphogenous metastasis. The answers to this and other questions concerning the establishment of therapeutic strategies will require further investigations.

## V. Acknowledgments

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## VI. References

- Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M. and Gutkind, J. S. (2005) Prostaglandin E<sub>2</sub> promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science* 310; 1504–1510.
- Cipollone, F., Prontera, C., Pini, B., Marini, M., Fazia, M., De Cesare, D., Iezzi, A., Uchino, S., Boccoli, G., Saba, V., Chiarelli, F., Cucurullo, F. and Mezzetti, A. (2001) Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E<sub>2</sub>-dependent plaque instability. *Circulation* 104; 921–927.
- Cohen, E. G., Almahmeed, T., Du, B., Golijanin, D., Boyle, J. O., Soslow, R. A., Subbaramaiah, K. and Dannenberg, A. J. (2003) Microsomal prostaglandin E synthase-1 is overexpressed in head and neck squamous cell carcinoma. *Clin. Cancer Res.* 9; 3425–3430.
- Cornetta, A. J., Russell, J. P., Cunnane, M., Keane, W. M. and Rothstein, J. L. (2002) Cyclooxygenase-2 expression in human thyroid carcinoma and Hashimoto's thyroiditis. *Laryngoscope* 112; 238–242.
- Golijanin, D., Tan, J. Y., Kazior, A., Cohen, E. G., Russo, P., Dalbagni, G., Auburn, K. J., Subbaramaiah, K. and Dannenberg, A. J. (2004) Cyclooxygenase-2 and microsomal prostaglandin E synthase-1 are overexpressed in squamous cell carcinoma of the penis. *Clin. Cancer Res.* 10; 1024–1031.
- Jabbour, H. N., Milne, S. A., Williams, A. R., Anderson, R. A. and Boddy, S. C. (2001) Expression of COX-2 and PGE synthase and synthesis of PGE<sub>2</sub> in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. *Br. J. Cancer* 85; 1023–1031.
- Kudo, I. and Murakami, M. (2005) Prostaglandin E synthase, a terminal enzyme for prostaglandin E<sub>2</sub> biosynthesis. *J. Biochem. Mol. Biol.* 38; 633–638.
- Maeta, H., Ohgi, S. and Terada, T. (2001) Protein expression of matrix metalloproteinase 2 and 9 and tissue inhibitors of metalloproteinase 1 and 2 in papillary thyroid carcinomas. *Virchows Arch.* 438; 121–128.
- Murakami, M. and Kudo, I. (2006) Prostaglandin E<sub>2</sub> synthase: a novel drug target for inflammation and cancer. *Curr. Pharm. Des.* 12; 943–954.
- Nikiforov, Y. E. (2002) RET/PTC rearrangement in thyroid tumors. *Endocr. Pathol.* 13; 3–16.
- Nose, F., Ichikawa, T., Fujisawa, M. and Okayasu, I. (2002) Up-regulation of cyclooxygenase-2 expression in lymphocytic thyroiditis and thyroid tumors. *Am. J. Clin. Pathol.* 117; 546–551.
- Okayasu, I., Fujiwara, M., Hara, Y., Tanaka, Y. and Rose, N. R. (1995) 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.
- Ott, R. A., McCall, A. R., McHenry, C., Jarosz, H., Armin, A., Lawrence, A. M. and Paloyan, E. (1987) The incidence of thyroid carcinoma in Hashimoto's thyroiditis. *Am. Surg.* 53; 442–445.
- Pai, R., Soreghan, B., Szabo, I. L., Pavelka, M., Baatar, D. and Tarnawski, A. S. (2002) Prostaglandin E<sub>2</sub> transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat. Med.* 8; 289–293.
- Puxeddu, E., Mitsutake, N., Knauf, J. A., Moretti, S., Kim, H. W., Seta, K. A., Brockman, D., Myatt, L., Millhorn, D. E. and Fagin, J. A. (2003) Microsomal prostaglandin E<sub>2</sub> Synthase-1 is induced by conditional expression of RET/PTC in thyroid PCCL3 cells through the activation of the MEK-ERK pathway. *J. Biol. Chem.* 278; 52131–52138.
- Seno, H., Oshima, M., Ishikawa, T., Oshima, H., Takaku, K., Chiba, T., Narumiya, S. and Taketo, M. M. (2002) Cyclooxygenase 2- and prostaglandin E(2) receptor EP(2)-dependent angiogenesis in Apc(Delta716) mouse intestinal polyps. *Cancer Res.* 62; 506–511.
- Sonoshita, M., Takaku, K., Sasaki, N., Sugimoto, Y., Ushikubi, F., Narumiya, S., Oshima, M. and Taketo, M. M. (2001) Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. *Nat. Med.* 7; 1048–1051.
- Tsujii, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M. and Dubois, R. N. (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 93; 705–716.
- van Rees, B. P., Sivula, A., Thorén, S., Yokozaki, H., Jakobsson, P. J., Offerhaus, G. J. and Ristimäki, A. (2003) Expression of microsomal prostaglandin E synthase-1 is in intestinal type gastric adenocarcinoma and in gastric cancer cell lines. *Int. J. Cancer* 107; 551–556.
- Wang, D., Wang, H., Shi, Q., Katkuri, S., Walhi, W., Desvergne, B., Das, S. K., Dey, S. K. and Dubois, R. N. (2004) Prostaglandin E (2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. *Cancer Cell* 6; 285–281.
- Wang, H. W., Hsueh, C. T., Lin, C. F., Chou, T. Y., Hsu, W. H., Wang, L. S. and Wu, Y. C. (2006) Clinical implications of microsomal prostaglandin E synthase-1 overexpression in human non-small-cell lung cancer. *Ann. Surg. Oncol.* 13; 1224–1234.
- Yoshimatsu, K., Golijanin, D., Paty, P. B., Soslow, R. A., Jakobsson, P. J., DeLellis, R. A., Subbaramaiah, K. and Dannenberg, A. J. (2001) Inducible microsomal prostaglandin E synthase is overexpressed in colorectal adenomas and cancer. *Clin. Cancer Res.* 7; 3971–3976.