Biochemical and Biophysical Research Communications 430 (2013) 1078–1082

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The transcription factor Snail expressed in cutaneous squamous cell carcinoma induces epithelial–mesenchymal transition and down-regulates COX-2

Mitsuyoshi Shimokawa [a,b], Misako Haraguchi [a], Wakako Kobayashi [a], Yuko Higashi [b], Shigeto Matsushita [b], Kazuhiro Kawai [b], Takuro Kanekura [b], Masayuki Ozawa [a,*]

[a] Department of Biochemistry and Molecular Biology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan
[b] Department of Dermatology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

ARTICLE INFO

Article history:
Received 4 December 2012
Available online 19 December 2012

Keywords:
Snail
E-cadherin
COX-2
Cutaneous squamous cell carcinoma

ABSTRACT

Cutaneous spindle cell squamous cell carcinoma (SCC) is a rare, but highly malignant variant of SCC. The presence of spindle-shaped cells with a sarcomatous appearance, which are derived from squamous cells, suggests that these cells are produced as a result of epithelial–mesenchymal transition (EMT). EMT is a complex process in which epithelial cells lose their polarity and cell–cell contacts, while also acquiring increased motility and invasiveness. Snail regulates EMT by binding to proximal E-boxes in the promoter region of E-cadherin and repressing its transcription. When examining the expression of EMT markers and Snail in spindle cell SCCs, we found that cyclooxygenase-2 (COX-2) expression was down-regulated. Since it has been shown that COX-2 is constitutively overexpressed in a variety of malignancies, including colon, gastric, and lung carcinomas, the down-regulation of COX-2 expression was unexpected. The presence of E-box-like sequences in the promoter region of COX-2 prompted us to perform a more detailed analysis. We introduced a Snail expression vector into keratinocyte-derived cell lines (HaKaT, HSC5, and A431 cells), and isolated stable transfectants. We determined that COX-2 expression was down-regulated in cells expressing Snail. Consistent with these observations, reporter assays revealed that COX-2 promoter activity was repressed upon Snail overexpression. Thus Snail down-regulates COX-2 in these cells.

1. Introduction

Cutaneous spindle cell squamous cell carcinoma (SCC) is a rare, but highly malignant variant of SCC that was first described by Martin and Stewart in 1935 [1]. Histologically, spindle cell SCC infiltrates the dermis as single, atypical spindle cells with elongated nuclei, and unlike conventional SCC, there are typically no signs of keratinization [2]. The presence of spindle-shaped cells with a sarcomatous appearance, which are derived from squamous cells, suggests that they are produced as a result of epithelial–mesenchymal transition (EMT).

EMT is a complex process by which epithelial cells lose their polarity and reorganize their cytoskeleton, while also acquiring a mesenchymal phenotype and increased motility [3,4]. In addition to tissue remodeling, organ development, and wound healing, EMT plays a critical role in cancer progression and organ fibrosis [4–7]. Loss of a polarized epithelial cell phenotype and acquisition of mesenchymal characteristics endow tumor cells with the potential to invade and metastasize. Snail is a regulator of EMT that represses E-cadherin transcription by binding to proximal E-boxes (a 5′-CACCTG-3′ sequence) in the promoter regions of target genes [8,9]. Correlative studies have shown that there is an inverse relationship between E-cadherin expression and Snail expression in human samples [10].

During examination of the expression of E-cadherin and vimentin, which are EMT markers, and Snail, an EMT inducer, in spindle cell SCCs, we found down-regulation of cyclooxygenase-2 (COX-2). COX-2 and its metabolite prostaglandin E2 (PGE2) play important roles in regulating diverse cellular functions under physiologic and pathologic conditions [11–14]. COX-2, the inducible isoenzyme, is constitutively overexpressed in a variety of malignancies, including colon, gastric, esophageal, prostate, pancreatic, breast, and lung carcinomas [13–17]. Therefore, the observed down-regulation of COX-2 expression was unexpected. The expression of Snail in spindle SCC and the presence of E-box-like sequences (CANNTG) in the promoter region of COX-2 gene suggested that Snail is responsible for the down-regulation of COX-2 in spindle SCC. In the present study, we wanted to determine whether Snail down-regulates COX-2 or not. We introduced a Snail expression vector into keratinocyte-derived cell lines and isolated stable transfectants. We obtained evidences showing that COX-2

Abbreviations: COX-2, cyclooxygenase-2; EMT, epithelial–mesenchymal transition; SCC, squamous cell carcinoma.

* Corresponding author. Fax: +81 99 264 5618.
E-mail address: mozawa@m.kufm.kagoshima-u.ac.jp (M. Ozawa).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.035

expression was down-regulated in Snail-expressing cells. Consistent with these observations, reporter assays revealed that COX-2 promoter activity was repressed upon Snail overexpression. These results provide molecular basis on our observation that COX-2 is down-regulated in spindle SCCs.

2. Materials and methods

2.1. Immunohistochemical staining

Immunohistochemical studies examining E-cadherin, vimentin, Snail, and COX-2 were performed on six formalin-fixed, paraffin-embedded specimens of cutaneous spindle cell SCC and three SCC specimens without spindle cells (non-spindle SCC) that had been collected and diagnosed at the Department of Dermatology, Kagoshima University Graduate School of Medical and Dental Sciences between 1990 and 2010. Tissue sections with 6 μm thickness were prepared from formalin-fixed, paraffin-embedded specimens. Each section was deparaffinized in xylene, and antigenic activity was retrieved by incubating in 0.01 M citrate buffer (pH 6.0) in an autoclave (120 °C, 5 min). The sections were incubated with 3% H_2O_2 in methanol for 5 min to block endogenous peroxidase activity and then with 5% normal serum in phosphate-buffered saline (PBS) for 30 min to block non-specific antibody binding. Each section was subsequently incubated (1 h, room temperature) with a primary antibody; followed by normal serum, and then exposed for 1 h to biotinylated horse secondary antibodies (Vector Laboratories, Burlingame, CA, USA) diluted 1:100 in PBS. After a 30 min reaction with an avidin–biotin peroxidase complex (Vector Laboratories), the samples were incubated with diaminobenzidine (DAB) (Vector Laboratories).

2.2. Cells and transfection

Human cutaneous SCCs, HSC5 and A431 cells, and human precancerous keratinocyte HaCaT cells were grown in DME supplemented with 10% FCS. These cells (2×10^5) were transfected with 10 μg of an expression vector encoding HA-tagged human Snail (pC-SnailHA) or a control vector (pCAG-neo) using the calcium phosphate method as previously described [18]. Stable transfectants were isolated after selecting with G418, and the expression of HA-tagged Snail was determined by immunofluorescence staining and immunoblot analysis using an anti-HA mAb. Isolated clones were designated as HSC5-Snail, HSC5-Neo, HaCaT-Snail, and HaCaT-Neo cells. A431-Neo and A431-Snail cells were previously described [18].

2.3. Antibodies

Mouse mAbs against E-cadherin and its FITC derivative were purchased from BD Biosciences (Lexington, KY, USA). A mouse mAb against vinculin was purchased from Sigma (St Louis, MO, USA). A mouse mAb against Snail was purchased from CST Japan. A goat pAb against COX-2 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and used for immunohistochemistry. A rat mAb against HA and mouse mAb against vimentin were purchased form Roche Diagnostics GmbH (Mannheim, Germany) and Zymed Laboratories (South San Francisco, CA, USA), respectively. For immunohistochemical staining, a rabbit pAb against Snail was obtained from Abgent (San Diego, CA, USA).

2.4. Fluorescence microscopy

Immunofluorescence labeling of cells was performed as previously described [19]. Briefly, cells were fixed with 3% paraformaldehyde in PBS for 20 min at room temperature. The cells were permeabilized with 0.1% Triton X-100 and then incubated with primary and secondary antibodies. The stained cells were analyzed using a conventional Olympus fluorescence microscope (Tokyo, Japan) equipped with an Olympus DP73 digital camera.

2.5. Immunoblotting

For immunoblot analysis, cells were boiled for 5 min in SDS gel sample buffer. Proteins were separated by either 8% or 15% polyacrylamide gel electrophoresis, and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in PBS, and then incubated with specific primary antibodies followed by peroxidase-conjugated secondary antibodies (Jackson Immuno Research Laboratories, West Grove, PA, USA). After washing with PBS containing 0.1% Tween-20, protein bands were visualized by enhanced chemiluminescence (ECL) (Amersham International, Little Chalfont, UK).

2.6. RT-PCR analysis

Total RNA was extracted from the cells using the Isogen kit (Wako, Osaka, Japan) and then reverse transcribed using Rever Tra Ace (Toyobo, Osaka, Japan). The resulting cDNAs were used as templates for specific PCR reactions using GoTaq DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were optimized for each primer pair as previously described [20]. The following primer combinations were used: COX-2, sense (GGTCTGGTGCCTGGTCTGATGATG) and antisense (GTCCTTTCAAGGAGAATGGTGC); β-actin, sense (CAAAGACCTGTACGCCAACAC) and antisense (CATACTCCTGCTTGCTGATCC).

2.7. Reporter assay

The luciferase reporter construct containing the human E-cadherin promoter (−178 to +66) was previously described [18]. Luciferase reporter constructs containing the full-length COX-2 promoter DNA (−1432/+59 bp) or its truncated fragments [21] were provided by Dr. L.R. Ballou (Department of Veterans Affairs Medical Center). The assays were performed as previously described [18]. Briefly, HEK293T cells (1.5×10^5) were seeded in 35 mm plates for 24 h prior to transfection. The reporter constructs (0.5 μg) were transfected into cells together with the pCAG vector containing Snail cDNA or empty pCAG vector (2 μg), and 40 ng of pRL-CMV vector using Lipofectamine Plus. After 48 h, both firefly (FL) and Renilla luciferase (RL) activities were measured using a Dual luciferase reporter assay kit (Promega). FL activity was normalized to the RL activity. The experiments were performed in triplicate [18].

3. Results

3.1. Spindle cell SCC exhibits mesenchymal features and down-regulation of COX-2

Hematoxylin and eosin staining of cutaneous spindle cell SCC specimens showed undifferentiated spindle-shaped, fibroblast-like cells (Fig. 1A). To confirm the possible occurrence of EMT in spindle cell SCC, we stained specimens of spindle cell SCC and SCC without spindle cells (non-spindle SCC) with E-cadherin and vimentin antibodies (Fig. 1B). Staining with an E-cadherin antibody revealed a honeycomb pattern in non-spindle SCC but significantly reduced staining in spindle cell SCC. However, there was little or no vimentin or Snail expression in non-spindle SCC. Spindle cell SCC showed cytoplasmic staining for vimentin and nuclear staining of Snail.

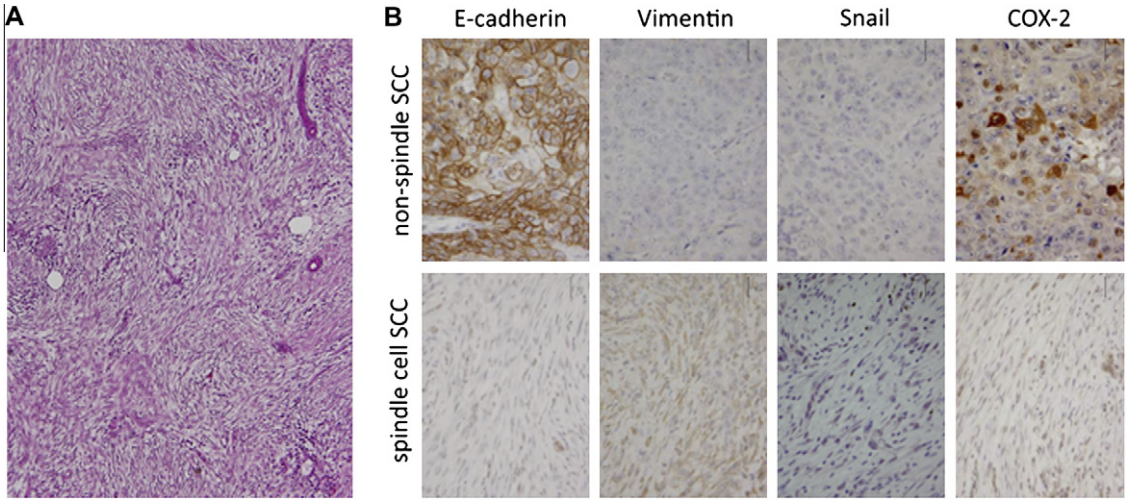

Fig. 1. Expression of E-cadherin, vimentin, Snail, and COX-2 in non-spindle SCC and spindle cell SCC. (A) Spindle cell SCC exhibiting undifferentiated spindle-shaped, fibroblast-like cells [hematoxylin and eosin stain (H&E); original magnification ×200]. (B) E-cadherin was detected in non-spindle SCC in a honeycomb pattern, but not in spindle cell SCC. There was little to no staining of vimentin or Snail in non-spindle SCC. Although COX-2 was expressed in some but not all non-spindle SCC, it was significantly reduced in spindle cell SCC. Among six specimens of cutaneous spindle cell SCC and three specimens of non-spindle SCC, the representative results of each SCC are shown.

These data strongly suggest that spindle cell SCC occurred as a result of EMT in SCC of epithelial nature. Interestingly, we observed that COX-2 was down-regulated in spindle cell SCC; although COX-2 expression was observed in some but not all of non-spindle SCC, it was significantly reduced in spindle cell SCC (Fig. 1B).

3.2. Expression of Snail in human epidermal cancer cells results in COX-2 down-regulation

The data presented above suggested that EMT induced upon Snail expression may be related to COX-2 down-regulation. To examine this possibility, we established stable Snail transfectants using cutaneous SCC cell lines (HSC5 and A431 cells) and precancerous keratinocytes (HaCaT cells). Expression of Snail in HSC5 cells induced morphological changes; the cells became fibroblastic (Fig. 2A), and were not stained with an anti-E-cadherin antibody (Fig. 2B). Snail expression in HaCaT cells also induced morphological changes (data not shown), and these cells were not stained with an anti-E-cadherin antibody (Fig. 2B). We previously showed that ectopic expression of Snail in A431 cells induced EMT, in which the cells change from an epithelial to fibroblastic morphology, and the down-regulation of E-cadherin [18]. Immunoblot analysis revealed that Snail expression in A431, HSC5, and HaCaT cells reduced E-cadherin expression and induced vimentin expression (Fig. 3A), and thus these changes can be classified as EMT.

Consistent with our observations of clinical specimens in which spindle cell SCC expressing Snail showed reduced COX-2 expression, stable Snail transfectants also had lower COX-2 expression based on immunoblot analyses (Fig. 3A). RT-PCR analysis revealed virtually no COX-2 mRNA in stable Snail transfectants of A431 cells (Fig. 3B). Thus, Snail down-regulates COX-2 at the transcriptional level.

3.3. The effects of Snail on the activity of the COX-2 promoter

To further confirm that Snail down-regulates COX-2 at the transcriptional level, we performed reporter assays. We transfected reporter constructs containing the COX-2 promoter (Fig. 4A) into

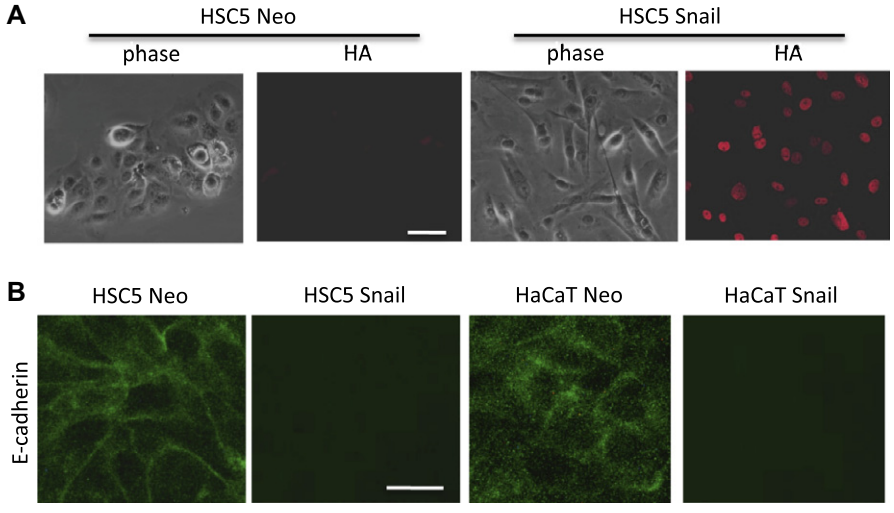

Fig. 2. Expression of Snail in human epidermal cancer cells results in EMT. (A) Phase contrast microscopic observation reveals morphological changes induced by Snail expression. Immunofluorescence staining with anti-HA antibodies shows the nuclear localization of the Snail protein in HSC5 cells transfected with an HA-tagged Snail expression vector. Bar, 50 μm. (B) Staining with E-cadherin antibodies shows that HSC5 cells or HaCaT cells transfected with a control vector (HSC5 Neo and HaCaT Neo) are positive for E-cadherin while cells expressing Snail (HSC5 Snail and HaCaT Snail) are negative. Bar, 50 μm.

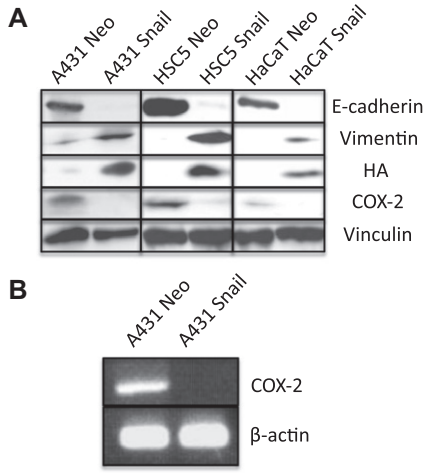

Fig. 3. Expression of Snail in human epidermal cancer cells results in decreased COX-2 expression. (A) Immunoblot analysis of stable Snail transfectants. Proteins were detected with the indicated antibodies in order to assess changes in the expression of proteins involved in EMT. Stable Snail transfectants (A431 Snail, HSC5 Snail, and HaCaT Snail) and control transfectants (A431 Neo, HSC5 Neo, and HaCaT Neo) were lysed in SDS sample buffer and subjected to immunoblot analysis. HA-tagged Snail protein was detected with anti-HA antibodies. E-cadherin and COX-2 expression was down-regulated, while that of vimentin was increased in Snail-expressing cells. Vinculin serves as an internal protein loading control. (B) RT-PCR analysis of COX-2 mRNA in A431 Neo and A431 Snail cells. β-Actin was used as an internal control.

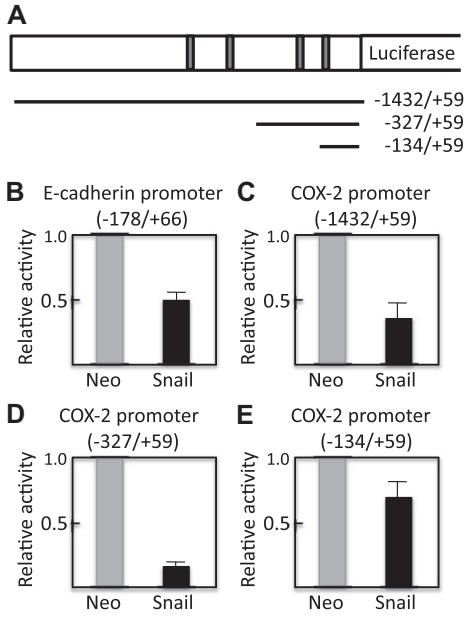

Fig. 4. Snail-induced down-regulation of COX-2 expression is attributed to decreased promoter activity. (A) The promoter region of human COX-2 in which four possible Snail-binding sites (CANNTG) in positions −525 to −520, −427 to −422, −188 to −183, and −134 to −129 are indicated by shadowed boxes. The putative transcription start point is denoted by +1 [19]. The full-length and two deletion constructs used for the assays are shown. Snail-mediated repression of the promoter activities of human E-cadherin (B) and COX-2 (C) in HEK293T cells. Reporter constructs carrying the promoters for human E-cadherin or COX-2 were co-transfected with the pCAG-Snail vector or empty pCAGneo vector. The promoter activities of E-cadherin and COX-2 were repressed upon Snail expression. Although a deletion construct containing the two proximal binding sites (−327 to +59) was repressed by Snail (D), another deletion construct containing only the most proximal binding site (−134 to +59) was not repressed by Snail (E), indicating that this site (−188 to −183) is likely the Snail-binding site. The results are represented as the mean ± SD of three independent experiments.

HEK293T cells and analyzed promoter activity. Consistent with the RT-PCR analysis, the activity of the construct containing the COX-2 promoter (position numbers −1432 to +59) was repressed upon Snail expression in a manner similar to that for E-cadherin (position numbers −178 to +66) (Fig. 4B and C). This promoter region contains four putative Snail-binding sequences (CANNTG). A deletion construct containing the two proximal binding sites (−327 to +59) was repressed by Snail expression (Fig. 4D). However, another deletion construct containing only the most proximal binding site (−134 to +59) was not repressed by Snail expression (Fig. 4E), indicating that this site (−188 to −183) is likely the Snail-binding site.

4. Discussion

In the present study, we showed that cutaneous spindle cell SCC has characteristics of cancer cells that have undergone EMT. While non-spindle SCC (SCC without spindle cells) expressed E-cadherin in a honeycomb pattern and did not exhibit vimentin staining, spindle cell SCC had no or significantly reduced E-cadherin expression and was positive for vimentin staining. Since these changes, such as loss of epithelial and gain of mesenchymal markers, are hallmarks of EMT [4–6], cutaneous spindle cell SCC can be classified as cells that have undergone EMT. Consistent with our observations, previous papers reported that cutaneous spindle cell SCC is positive for vimentin [22] and negative for some but not all types of cytokeratins, such as AE1/3 or K903 [23]. Since these studies did not examine both epithelial and mesenchymal markers, to the best of our knowledge, our study is the first to show that EMT occurs in cutaneous spindle cell SCC. We further demonstrate that Snail, an EMT inducer, is expressed in cutaneous spindle cell SCC.

Ectopic expression of Snail in HSC5 or A431 cells, human SCC cell lines, or in HaCaT cells, immortalized human keratinocytes, induced changes that are characteristic of EMT. The observed changes included the acquisition of a fibroblast-like morphology and decreased expression of E-cadherin at sites of cell–cell contact. Immunoblot analysis showed significantly reduced E-cadherin expression and increased vimentin expression. In addition, the SCC cell lines A431 and HSC5 express higher amounts of the COX-2 protein compared to HaCaT cells, a precancerous keratinocyte line, but this COX-2 expression was down-regulated by Snail. RT-PCR analyses also revealed decreased COX-2 mRNA levels in A431 cells expressing Snail. Thus, Snail expression decreased COX-2 expression at the transcriptional level. Consistent with these findings, the activity of the reporter construct containing the COX-2 promoter was repressed in the presence of Snail.

COX-2 is frequently up-regulated in various cancers, and elevated COX-2 expression is associated with tumor invasion, metastasis, and a poor prognosis in non-small cell lung cancer [24]. Since its expression is tightly coupled to cellular activity, rapid in onset, and transient, COX-2 can be defined as an immediate early gene [25]. A recent study showed that COX-2 and PGE2 expression in lung cancer cells results in a significant reduction in E-cadherin through a mechanism that requires the transcription factors ZEB1 and Snail, and that inhibition of COX-2 rescues E-cadherin expression [26]. Furthermore, Snail has been reported to increase COX-2 expression [27]. In addition to WSU-HN13 cells (with which 18-fold increase of COX-2 mRNA by Snail was observed), Lyons et al. [27] found 1.93-fold and 3.66-fold increase of COX-2 mRNA by Snail with A431 and HaCaT cells (cell lines used in our study). Our current results, however, show that Snail down-regulates COX-2 expression. The reason for this discrepancy is unknown. One possible explanation for these apparent contradictory findings might be that different Snail constructs were used. We used HA-tagged Snail, whereas Lyons et al. [27] used a fusion protein with the mutant estrogen receptor hormone-binding domain. To repress

E-cadherin promoter, Snail needs to interact with histone deacethylase and corepressor mSin3A [28]. Furthermore, the interactions of Snail with transcription regulators such as CtBP and p300 are critical for the action of Snail [29]. Compared with HA tag, the estrogen receptor hormon-binding domain (36 kDa) is very large. Therefore, the presence of the domain may have some influence on these interactions and give different results. It has previously been shown that the addition of the hormone-binding domain to proteins has profound effects on the activity of transcription factors such as c-Fos [30].

Contrary to the observations mentioned above, a large body of experimental work indicates that PGE2 functions as an antifibrotic factor and that reduced PGE2 levels contribute to the pathogenesis of fibrosis [31,32]. In cultured lung fibroblasts, PGE2 expression results in suppressed cell proliferation [33], collagen production [32,34], and fibroblast-to-myofibroblast transition [35]. Furthermore, a recent study indicated that PGE2 potently inhibits EMT in renal epithelial cells and that COX-2-derived PGE2 partially mediates the antifibrotic effects of HGF [36]. If PGE2 has inhibitory effects on EMT, it seems reasonable that Snail, an EMT inducer, down-regulates COX-2, whose metabolite is a potent inhibitor of EMT.

Immunohistochemical staining of non-spindle SCC revealed heterogeneity in COX-2 expression, i.e., some cells were strongly stained while others were weakly stained with COX-2 antibodies. Thus, the environment, such as stroma cells surrounding carcinoma cells and the cytokines within the intercellular spaces, may strongly influence COX-2 expression. Cell context-dependent expression and transient nature of COX-2 expression should also be considered. Thus, additional studies are required to learn more about the mechanisms that regulate COX-2 expression.

Acknowledgments

This work was supported by the following grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan: Grants-in-Aid for Science Research Category C to M. O. and T. K. We thank Atsunori Baba and Mitsuhiro Hisadome for their support. We thank Tomoko Fukushige and Kanayo Gunshin for their excellent technical assistance and Kumiko Sato for secretarial assistance. We would like to thank the Joint Research Laboratory at Kagoshima University Graduate School of Medical and Dental Sciences for the use of their facilities.

References

[1] H.E. Martin, F.W. Stewart, Spindle cell epidermoid carcinoma, Am. J. Cancer 24 (1935) 273–297.
[2] M.B. Morgan, C. Purohit, T.R. Anglin, Immunohistochemical distinction of cutaneous spindle cell carcinoma, Am. J. Dermatopathol. 30 (2008) 228–232.
[3] E.D. Hay, An overview of epithelio–mesenchymal transformation, Acta Anat. (Basel) 154 (1995) 8–20.
[4] J.P. Thiery, Epithelial–mesenchymal transitions in tumour progression, Nat. Rev. Cancer 2 (2002) 442–454.
[5] M.A. Huber, N. Kraut, H. Beug, Molecular requirements for epithelial–mesenchymal transition during tumor progression, Curr. Opin. Cell Biol. 17 (2005) 548–558.
[6] J.J. Christiansen, A.K. Rajasekaran, Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis, Cancer Res. 66 (2006) 8319–8326.
[7] A. Nawshad, D. LaGamba, A. Polad, E.D. Hay, Transforming growth factor-beta signaling during epithelial–mesenchymal transformation: implications for embryogenesis and tumor metastasis, Cells Tissues Organs 179 (2005) 11–23.
[8] M.A. Nieto, The snail superfamily of zinc-finger transcription factors, Nat. Rev. Mol. Cell Biol. 3 (2002) 155–166.
[9] H. Peinado, D. Olmeda, A. Cano, Snail, Zeb, and bHLH factors in tumour progression: an alliance against the epithelial phenotype?, Nat Rev. Cancer 7 (2007) 415–428.
[10] C. Come, F. Magnino, F. Bibeau, P. De Santa Barbara, et al., Snail and slug play distinct roles during breast carcinoma progression, Clin. Cancer Res. 12 (2006) 5395–5402.
[11] J.R. Brown, R.N. DuBois, Cyclooxygenase as a target in lung cancer, Clin. Cancer Res. 10 (2004) 4266s–4269s.
[12] T. Kanekura, S.J. Lauledeerkind, K. Kirtikara, S. Goorha, L.R. Ballou, Cholecalciferol induces prostaglandin E_2 biosynthesis and transglutaminase activity in human keratinocytes, J. Invest. Dermatol. 111 (1998) 634–639.
[13] S.M. Dubinett, S. Sharma, M. Huang, M. Dohadwala, M. Pold, J.T. Mao, Cyclooxygenase-2 in lung cancer, Prog. Exp. Tumor Res. 37 (2003) 138–162.
[14] A.J. Dannenberg, K. Subbaramaiah, Targeting cyclooxygenase-2 in human neoplasia: rationale and promise, Cancer Cell 4 (2003) 431–436.
[15] A.J. Dannenberg, D. Zakim, Chemoprevention of colorectal cancer through inhibition of cyclooxygenase-2, Semin. Oncol. 26 (1999) 499–504.
[16] M. Huang, M. Stolina, S. Sharma, et al., Non-small cell lung cancer cyclooxygenase-2 dependent regulation of cytokine balance in lymphocytes and macrophages: upregulation of interleukin 10 and down-regulation of interleukin 12 production, Cancer Res. 58 (1998) 1208–1216.
[17] Y. Higashi, T. Kanekura, T. Kanzaki, 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 (2000) 667–671.
[18] M. Ohkubo, M. Ozawa, The transcription factor Snail downregulates the tight junction components independently of E-cadherin downregulation, J. Cell Sci. 117 (2004) 1675–1685.
[19] Y. Miyashita, M. Ozawa, A dileucine motif in its cytoplasmic domain directs β-catenin-uncoupled E-cadherin to the lysosome, J. Cell Sci. 120 (2007) 4395–4406.
[20] M. Haraguchi, T. Okubo, Y. Miyashita, Y. Miyamoto, et al., Snail regulates cell-matrix adhesion by regulation of the expression of integrins and basement membrane proteins, J. Biol. Chem. 283 (2008) 23514–23523.
[21] K. Kirtikara, R. Raghow, S.J. Lauledeerkind, S. Goorha, T. Kanekura, L.R. Ballou, Transcriptional regulation of cyclooxygenase-2 in the human microvascular endothelial cell line, HMEC-1: control by the combinatorial actions of AP2, NF-IL-6 and CRE elements, Mol. Cell Biochem. 203 (2000) 41–51.
[22] J.E. Dotto, E.J. Glusac, P63 is a useful marker for cutaneous spindle cell squamous cell carcinoma, J. Cutan. Pathol. 33 (2006) 413–417.
[23] J.E. Sigel, M. Skacel, W.F. Bergfeld, N.S. House, M.S. Rabkin, J.R. Goldblum, The utility of cytokeratin 5/6 in the recognition of cutaneous spindle cell squamous cell carcinoma, J. Cutan. Pathol. 28 (2001) 520–524.
[24] O.N. Tucker, A.J. Dannenberg, E.K. Yang, F. Zhang, L. Teng, J.M. Daly, R.A. Soslow, J.L. Masferrer, B.M. Woerner, A.T. Koki, T.J. Fahey III, Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer, Cancer Res. 59 (1999) 987–990.
[25] H.R. Herschman, Primary response genes induced by growth factors and tumor promoters, Annu. Rev. Biochem. 60 (1991) 281–319.
[26] M. Dohadwala, S.C. Yang, J. Luo, S. Sharma, R.K. Batra, M. Huang, Y. Lin, L. Goodglick, K. Krysan, M.C. Fishbein, L. Hong, C. Lai, R.B. Cameron, R.M. Gemmill, H.A. Drabkin, S.M. Dubinett, Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin E(2) induces transcriptional repressors ZEB1 and snail in non-small cell lung cancer, Cancer Res. 66 (2006) 5338–5345.
[27] J.G. Lyons, V. Patel, N.C. Roue, S.Y. Fok, L.L. Soon, G.M. Halliday, J.S. Gutkind, Snail up-regulates proinflammatory mediators and inhibits differentiation in oral keratinocytes, Cancer Res. 68 (2008) 4525–4530.
[28] H. Peinado, E. Ballestar, M. Esteller, A. Cano, Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex, Mol. Cell Biol. 24 (2004) 306–319.
[29] C. Peña, J.M. García, V. García, J. Silva, G. Domínguez, R. Rodríguez, C. Maximiano, A. García de Herreros, A. Muñoz, F. Bonilla, The expression levels of the transcriptional regulators p300 and CtBP modulate the correlations between SNAIL, ZEB1, E-cadherin and vitamin D receptor in human colon carcinomas, Int. J. Cancer 119 (2006) 2098–2104.
[30] M. Schuermann, G. Hennig, R. Müller, Transcriptional activation and transformation by chimaeric Fos-estrogen receptor proteins: altered properties as a consequence of gene fusion, Oncogene 8 (1993) 2781–2790.
[31] R.J. Hodges, R.G. Jenkins, C.P. Wheeler-Jones, D.M. Copeman, S.E. Bottoms, G.J. Bellingan, C.B. Nanthakumar, G.J. Laurent, S.L. Hart, M.L. Foster, R.J. McAnulty, Severity of lung injury in cyclooxygenase-2-deficient mice is dependent on reduced prostaglandin E(2) production, Am. J. Pathol. 165 (2004) 1663–1676.
[32] R.J. McAnulty, N.A. Hernandez-Rodriguez, S.E. Mutsaers, R.K. Coker, G.J. Laurent, Indomethacin suppresses the anti-proliferative effects of transforming growth factor-beta isoforms on fibroblast cell cultures, Biochem. J. 321 (1997) 639–643.
[33] A. Fine, R.H. Goldstein, The effect of PGE2 on the activation of quiescent lung fibroblasts, Prostaglandins 33 (1987) 903–913.
[34] R.H. Goldstein, P. Polgar, The effect and interaction of bradykinin and prostaglandins on protein and collagen production by lung fibroblasts, J. Biol. Chem. 257 (1982) 8630–8633.
[35] J.E. Kolodsick, M. Peters-Golden, J. Larios, G.B. Toews, V.J. Thannickal, B.B. Moore, Prostaglandin E2 inhibits fibroblast to myofibroblast transition via E. prostanoid receptor 2 signaling and cyclic adenosine monophosphate elevation, Am. J. Respir. Cell Mol. Biol. 29 (2003) 537–544.
[36] A. Zhang, M.H. Wang, Z. Dong, T. Yang, Prostaglandin E2 is a potent inhibitor of epithelial-to-mesenchymal transition: interaction with hepatocyte growth factor, Am. J. Physiol.-Renal Physiol. 291 (2006) F1323–F1331.