Expression Pattern of Smad4/GATA3 as a Predictor of Survival in Invasive Ductal Carcinoma of the Breast

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Keywords

Smad4 protein · GATA3 protein · Prognosis · Breast cancer

Abstract

Background: Smad4 and GATA3 proteins are known prognostic markers in various cancers. Smad4 is a mediator linked to both tumour suppression and progression. GATA3 is a regulator of development and morphogenesis of the mammary gland. We assessed and compared the predictive performance of Smad4 and GATA3 for clinical outcomes in patients with breast cancer. **Methods:** The combined expression pattern based on Smad4+/- and GATA3+/- was evaluated by immunostaining using breast cancer tissue microarray, and the relationships between protein expression and clinicopathological variables were analysed. **Results:** Smad4 expression was only associated with an ill-defined tumour border, whereas GATA3 was associated with several good prognostic fac-

tors. On analysis of combined markers, there was a significant difference in the expression of fascin (an important factor for cancer invasiveness) between the Smad4+/GATA3- and Smad4-/GATA3+ groups. Smad4+/GATA3- was correlated with worse clinicopathological parameters, relapse-free survival (RFS), and overall survival (OS), compared to Smad4-/GATA3+. **Conclusion:** Combined markers of Smad4/GATA3 showed a superior performance compared to single markers for predicting RFS and OS in patients with breast cancer.

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Introduction

Breast cancer accounts for 23% of total cancer cases and is the most common cause of cancer death among females [1]. According to the hormonal status, histological type, and American Joint Committee on Cancer

(AJCC) stage, patients with breast cancer receive appropriate treatment such as chemotherapy, radiotherapy, and surgery. Recently, molecular techniques have facilitated the identification of tumour markers that can increase diagnostic performance, including tumour classification, in addition to predicting prognosis and therapeutic response [2, 3]. Differential expression of various proteins has contributed to the development of more efficient treatment options [4].

Several studies using different biomarkers have revealed relationships with clinical parameters and prognosis in breast cancer [5, 6]. Invasive ductal carcinoma (IDC) is the most common type of breast cancer and is characterised by profound heterogeneity [7]. However, considering the multifactorial origin and diversity of breast cancers, prognostic models based on a specific marker are still debated. Therefore, the use of combined markers based on molecular mechanisms may provide greater insight into the development and progression of malignancy and clinical outcomes [8, 9].

The tumour suppressor Smad4 belongs to the family of downstream mediators of transforming growth factor (TGF) and is an important multifunctional cytokine that regulates cell proliferation, differentiation, extracellular matrix production, and the development of carcinoma [10]. Several studies have demonstrated that Smad4 inactivation is significantly correlated with tumourigenesis, especially in pancreatic [11] and colorectal [12] cancer. Various proteins associated with Smad4 have been reported, but the regulatory mechanisms involving Smad4-interacting proteins remain unclear. A recent study demonstrated that GATA3 abrogated TGF- β -Smad signalling by abolishing the interactions between Smad4 and DNA elements linked to fascin function [9]. GATA3 is a zinc finger transcription factor that regulates tumour growth inhibition as well as the development and morphogenesis of the mammary glands [13]. In contrast, fascin, as a prometastasis actin bundling protein, is a predictor of relatively worse prognosis in breast cancer [14]. On the basis of *in vitro* experimental results [9], we hypothesised that GATA3 expression would reduce Smad4 expression and thereby also inhibit fascin expression in human breast cancer. Combined assessment of Smad4 and GATA3 expression provides more detailed information for predicting clinical outcomes in breast cancer than expression of either marker alone. The aim of this study was to investigate the expression patterns of Smad4 and GATA3, as well as the Smad4-mediated fascin level, in patients with breast cancer. Furthermore, we evaluated whether combinatorial expression is associated with patient survival.

Materials and Methods

Patient Selection and Characteristics

Clinicopathological findings were collected from patient medical records for 226 cases diagnosed with IDC at Kangbuk SMC between 2000 and 2005. Treatments for IDC included modified radical mastectomy in 205 patients and breast-conserving surgery with axillary lymph node dissection in 21 patients. The clear boundaries of tumour borders on gross examination were referred to as “well-defined” while the blurry ones were referred to as “ill-defined.”

The histologic grade was determined according to the modified Bloom-Richardson-Elston grading system [15]. Tumours were staged with reference to the size and/or extent of tumour (T), regional lymph node involvement (N), and metastasis (M) using the 7th AJCC staging system. After surgery, 224 patients received adjuvant treatment such as chemotherapy, trastuzumab, or tamoxifen. Fifty-five patients developed local recurrence and/or new distant metastases. Forty-seven patients died during the follow-up period.

Tissue Microarray Construction

A series of tumour tissue microarray (TMA) specimens were assembled using a tissue-array instrument (AccuMac Arrayer; ISU ABXIS Co. Ltd., Seoul, Korea). Tumour TMAs consisted of 10 $\times$ 6 arrays of 2.0-mm tissue cores from representative paraffin blocks. Taking into account the limitations associated with obtaining representative areas of a tumour, we used duplicate 2.0-mm-diameter tissue cores from each donor block. The percentage of tumour in the tissue cores was >70%.

Immunohistochemical Staining and Scoring

Immunostaining with antibodies against human epidermal growth factor receptor 2 (HER2; 1:200; SP3 Clone; Labvision), oestrogen receptor (ER; 1:200; SP1 Clone; Labvision, Fremont, CA, USA), progesterone receptor (PR; 1:200; PgR636 Clone; Dako, Glostrup, Denmark), Ki-67 (1:500; MIB-1 Clone; Dako), and p53 (1:5,000; DO-7 Clone; Dako) was performed using a Dako Auto-stainer with a Universal Staining System (DakoCytomation, Carpinteria, CA, USA) and a ChemMate™ DAKO EnVision™ Detection Kit. Immunostaining of ER, PR, and HER-2 was assessed based on the American Society of Clinical Oncology/College of American Pathologists guidelines [16, 17]. Repeat testing was performed if results were discordant with other histopathological findings.

For this study, positive immunostaining with antibodies against GATA3 (1:100; L50-823; Cell Marque, Rocklin, CA, USA) and Smad4 (1:100; EP618Y; Abcam, Cambridge, UK) was defined as only nuclear staining regardless of the cytoplasmic staining. We used human lung carcinoma and urothelium (transitional epithelium), respectively, for positive controls of Smad4 and GATA3, while we adopted tonsil and placental tissue as negative controls.

Positivity for anti-fascin antibody (1:500; clone 55K-2; Dako) was defined as exclusive cytoplasmic staining without nuclear staining. The expression of Smad4 was graded according to both the intensity and percentage of positively stained tumour cells [18]. The intensity of nuclear staining was recorded separately as follows: 0 (no staining), 1 (weak), 2 (moderate), or 3 (strong). The extent of staining was graded as follows: 0 (0–5%), 1 (6–25%), 2 (26–50%), 3 (51–75%), or 4 (>75%; Fig. 1). The combined immunoreactive score (IRS) was generated by multiplying the score for

Fig. 1. Micrographs showing negative (a) and positive (b) Smad4 nuclear expression, and negative (c) and positive (d) GATA3 nuclear expression by immunohistochemical staining of breast cancer specimens. Original magnification. $\times 200$.

intensity by the score for extent. When a discrepancy occurred between duplicate cores, the higher score from the 2 tissue cores was used as the final score. Molecular subtypes were classified as follows: luminal A (ER+ and/or PR+, HER2-, Ki-67 <14%), luminal B HER2- (ER+ and/or PR+, HER2-, Ki-67 $\geq 14\%$), luminal B HER2+ (ER+ and/or PR+, HER2+, any Ki-67), HER2+ (ER- and PR-, HER2+), and triple-negative (ER-, PR-, and HER2-) [19, 20]. On the basis of published data, immunoreactivity for GATA3 was divided into “negative” (expression <50%) and “positive” (expression $\geq 50\%$) [21]. p53 defined nuclear staining of tumour cells as positive. Positive fascin immunostaining was defined as exclusive cytoplasmic staining with no nuclear staining.

We used receiver-operating characteristic (ROC) curves for determination of the optimal cut-off of Smad4. The cut-off value calculated using the ROC curve was used to evaluate the relationship between Smad4 and survival (area under the ROC: 0.594). On the basis of the ROC curve, Smad4 expression was classified as “negative” (IRS ≤ 5) or “positive” (IRS >5).

Statistical Analysis

Categorical variables were compared using the χ^2 test and linear by linear association test. Continuous variables such as p53 and fascin expression were compared using the Student *t* test. In sur-

vival analyses, plots were generated using the Kaplan-Meier curve and were compared using the log-rank test. Multivariate analysis was performed to identify independent prognostic markers for relapse-free survival (RFS) and overall survival (OS) using a Cox multistep regression model. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the R package (<http://www.r-project.org/>) and SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinicopathological Characteristics Associated with Smad4 and GATA3 Expression

Smad4 expression was associated with an ill-defined tumour border. GATA3 expression was correlated with good prognostic parameters such as young age, low histological grade, and the absence of vascular invasion and necrosis (all $p < 0.05$). ER+ and PR+ and HER2- was not linked to Smad4, but was significantly related to GATA3

Fig. 2. Fascin is highly expressed in the Smad4+/GATA3- group compared with the Smad4-/GATA3+ group ($p < 0.001$).

(all $p < 0.05$; online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000449428). The expression of p53 was higher in the Smad4+ group than in the Smad4- group, but the difference was not statistically significant ($p = 0.274$). p53 expression was higher in the GATA3- group than in the GATA3+ group ($p = 0.003$).

A comparison of Smad4 and GATA3 expression revealed an inverse relationship, but it was not statistically significant ($p = 0.621$). Comparison of molecular subgroups showed that Smad4 expression was more frequently observed in the luminal B HER2- and HER2+ subgroup than in the HER2+ group. GATA3 expression was rare in the HER2 and triple-negative subgroups compared with the other subgroups.

Comparison of Clinicopathological Parameters and Expression Pattern of Smad4/GATA3

Patients were divided into 4 groups according to the expression pattern of Smad4 and GATA3: Smad4-/GATA3+ ($n = 45$), Smad4+/GATA3- ($n = 55$), Smad4+/GATA3+ ($n = 71$), and Smad4-/GATA3- ($n = 55$).

On comparison of the Smad4-/GATA3+ and Smad4+/GATA3- groups, Smad4+/GATA3- was associated with a high histological grade, vascular and perineural invasion, tumour necrosis, ER- and PR-, HER2+, and high

Table 1. Correlation between clinicopathological parameters and Smad4 and GATA3 expression

Parameter	<i>n</i> = 100	Smad4-/GATA3+ (<i>n</i> = 45)	Smad4+/GATA3- (<i>n</i> = 55)	<i>p</i> value (χ^2 test)
Age				
≤50 years	60	29 (48.3)	31 (51.7)	0.412
>50 years	40	16 (40)	24 (60)	
T category				
1	41	16 (39)	25 (61)	0.799 ^a
2	53	28 (52.8)	25 (47.2)	
3	6	1 (16.7)	5 (83.3)	
N category				
0	48	21 (43.8)	27 (56.2)	0.803 ^a
1	30	16 (53.3)	14 (46.7)	
2	11	3 (27.3)	8 (72.7)	
3	11	5 (45.5)	6 (54.5)	
Tumour border				
Well defined	21	13 (61.9)	8 (38.1)	0.08
Ill defined	79	32 (40.5)	47 (59.5)	
Number of tumours				
Single	91	40 (44)	51 (56)	0.728 ^b
Multiple	9	5 (55.6)	4 (44.4)	
Histologic grade				
1	18	10 (55.6)	8 (44.4)	0.006^a
2	47	27 (57.4)	20 (42.6)	
3	35	8 (22.9)	27 (77.1)	
Lymphatic invasion				
Negative	51	26 (51)	25 (49)	0.22
Positive	49	19 (38.8)	30 (61.2)	
Vascular invasion				
Negative	93	45 (48.4)	48 (51.6)	0.016^b
Positive	7	0 (0)	7 (100)	
Perineural invasion				
Negative	85	42 (49.4)	43 (50.6)	0.035
Positive	15	3 (20)	12 (80)	
Tumour necrosis				
Absence	62	33 (53.2)	29 (46.8)	0.035
Presence	38	12 (31.6)	26 (68.4)	
Central tumour fibrosis				
Absence	84	37 (44)	47 (56)	0.661
Presence	16	8 (50)	8 (50)	
EIC				
Absence	85	37 (43.5)	48 (56.5)	0.482
Presence	15	8 (53.3)	7 (46.7)	
ER				
Negative	30	0 (0)	30 (100)	<0.001
Positive	70	45 (64.3)	25 (35.7)	
PR				
Negative	44	8 (18.2)	36 (81.8)	<0.001
Positive	56	37 (66.1)	19 (33.9)	
HER2				
Negative	77	39 (50.6)	38 (49.4)	0.038
Positive	23	6 (26.1)	17 (73.9)	
p53 expression, %		11.36	35.09	<0.001^c
Fascin expression, %		2.16	27.65	<0.001^c

Values in parentheses are percentages. EIC, extensive intraductal component; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2. *p* values <0.05 are shown in bold.

^a Linear by linear association.

^b Fisher exact test.

^c Student *t* test.

Table 2. Expression of Smad4 and GATA3 according to molecular subtype

Expression	Luminal A	Luminal B HER2–	Luminal B HER2+	HER2	Triple negative
Cases, <i>n</i>	116	8	29	32	41
Smad4–/GATA3+	37 (31.9)	2 (25)	6 (20.7)	–	–
Smad4+/GATA3–	15 (12.9)	3 (37.5)	7 (24.1)	10 (31.2)	20 (48.8)
Smad4+/GATA3+	51 (44)	2 (25)	12 (41.4)	5 (15.6)	1 (2.4)
Smad4–/GATA3–	13 (11.2)	1 (12.5)	4 (13.8)	17 (53.1)	20 (48.8)

Values in parentheses are percentages. HER2, human epidermal growth factor receptor 2.

Table 3. RFS and OS analyses according to combined markers of Smad4 and GATA3

	Univariate significance ^a	Multivariate significance ^b	Relative risk	95% CI
<i>RFS</i>				
Smad4–/GATA3+ vs. Smad4+/GATA3–	<0.001	0.003	7.141	1.95–26.15
AJCC stage (I or II vs. III)	0.028	0.227	1.744	0.708–4.297
Histological grade (1 or 2 vs. 3)	0.051	0.758	0.842	0.283–2.508
Lymphatic invasion (absence vs. presence)	0.084	0.636	1.276	0.465–3.497
Perineural invasion (absence vs. presence)	<0.001	0.01	3.171	1.313–7.662
Oestrogen receptor (absence vs. presence)	0.05	0.679	1.22	0.476–3.125
<i>OS</i>				
Smad4–/GATA3+ vs. Smad4+/GATA3–	0.001	0.04	9.509	1.109–81.557
AJCC stage (I or II vs. III)	0.073	0.508	1.478	0.464–4.707
Histological grade (1 or 2 vs. 3)	0.029	0.641	0.707	0.165–3.033
Lymphatic invasion (absence vs. presence)	0.118	0.723	1.277	0.331–4.934
Perineural invasion (absence vs. presence)	<0.001	0.018	4.025	1.274–12.716
Oestrogen receptor (absence vs. presence)	0.003	0.415	0.619	0.196–1.960

7th AJCC staging system. *p* values <0.05 are shown in bold.

^a log-rank test.

^b Cox proportional hazard model.

p53 expression (all *p* < 0.05; Table 1). Interestingly, fascin, a poor prognostic marker, was highly expressed in the Smad4+/GATA3– subgroup compared to Smad4–/GATA3+ (*p* < 0.001; Fig. 2). In a comparative analysis between the Smad4+/GATA3– and Smad4+/GATA3+ groups, Smad4+/GATA3– was correlated with old age, high histological grade, and ER– and PR– (all *p* < 0.05). In comparative analysis between the Smad4+/GATA3+ and Smad4–/GATA3– groups, Smad4+/GATA3+ was related to young age, ill-defined tumour border, low histological grade, absence of necrosis, ER+, and PR+ (all *p* < 0.05). In comparative analysis between the Smad4–/GATA3+ and Smad4+/GATA3+ or between Smad4–/

GATA3+ and Smad4–/GATA3– groups, there was a significant difference in tumour border. There was no significant difference in any parameters tested between the Smad4+/GATA3– and Smad4–/GATA3– groups.

In the comparison of molecular subtypes, Smad4–/GATA3+ was observed in the luminal A, luminal B HER2– and luminal B HER2+ subtypes, but was not detected in HER2 and triple-negative subtypes (Table 2).

Comparison between Survival and Expression Patterns of Smad4 and GATA3

Smad4 expression was associated with short RFS and OS (both *p* < 0.05). GATA3 expression was linked to long

Fig. 3. RFS (**a**) and OS (**b**) curves derived via the Kaplan-Meier method showing correlations with expression patterns of Smad4 (S) and GATA3 (G) (Smad4-/GATA3+ vs. Smad4+/GATA3-; all $p < 0.05$).

RFS ($p = 0.031$), but not to OS. In multivariate analyses (adjusted for AJCC stage, histological grade, lymphatic and perineural invasion, and ER), there was no relationship between OS and Smad4, but RFS remained associated with Smad4. GATA3 expression did not correlate with RFS (online suppl. Table 2).

In the comparison of the Smad4+/GATA3- and Smad4-/GATA3+ groups, Smad4+/GATA3- was significantly correlated with poor RFS and OS (both $p < 0.05$). In multivariate analyses, we still observed a significant correlation between Smad4+/GATA3- and poor RFS and OS (both $p < 0.05$; Table 3). In addition, there were significant differences in RFS and OS between the Smad4-/GATA3+ and Smad4+/GATA3+ groups and between the Smad4-/GATA3+ and Smad4-/GATA3- groups in the univariate analysis (all $p < 0.05$); however, these differences were not observed in the multivariate analysis (Fig. 3; online suppl. Table 3). In the luminal A subtype group, there was a difference in RFS between Smad4-/GATA3+ and Smad4+/GATA3- ($p = 0.02$), but little difference in OS (online suppl. Table 4).

Discussion

The aim of this study was to determine whether any relationship between the expression of Smad4 and GATA3 correlated with clinical outcomes in breast cancer. The Smad4 protein is involved in signal transduction from the cell surface to the nucleus. In the nucleus, a protein complex including Smad4 interacts with the nucleotides of DNA for transcriptional regulation and cell growth or proliferation [22]. GATA3 is necessary for mammary gland morphogenesis and for the maintenance of luminal cell differentiation [23].

Recently, a study by Sun et al. [9] demonstrated that GATA3 abrogated Smad4-mediated overexpression of fascin. Suppression of the canonical TGF- β -Smad signalling axis by GATA3 could potentially reduce active cellular migration and invasiveness in breast cancer cells. Fascin, the end product of the signalling axis, is related to cellular migration and invasion [24]. On the other hand, loss or low expression of GATA3 could promote fascin-induced invasiveness associated with poor clinical outcomes [25]. Therefore, it appears that Smad4, functioning

as a signal transduction mediator, plays an important role in the interaction between GATA3 and fascin. In neoplastic development, *Smad4* is a tumour suppressor gene that is mutated or deleted in 50% of pancreatic cancers [26] and loss of *Smad4* expression has been shown to be important for the progression of gastric [27], cervical [28], and colorectal [29] cancers. In addition, homozygous deletions of *Smad4* have been detected in approximately 8% of breast cancers [30]. However, *Smad4* is involved in many cell functions, such as gastrulation, differentiation, fibrosis, and extracellular matrix stimulation, according to different interacting genes [31–34]. Although *Smad4* mediates the regulation of various cellular functions for cancer progression [9, 35–37], most studies have only focused on the role of *Smad4* as a tumour suppressor.

Smad4 plays an important role as both a tumour suppressor and in the progression of breast cancer cells [37]. Previous studies reported that *Smad4* expression appears to be decreased in breast cancer relative to normal tissues and correlates with a good prognosis [38, 39]. However, one study suggested that patients with *Smad4*– tumours show a trend toward longer survival [40]. Another study demonstrated that loss of *Smad* signalling is correlated with a decrease in axillary lymph node metastasis [36]. Thus, it was questionable whether the role of *Smad4* as only a tumour suppressor could explain the opposing results of previous studies, especially in breast cancer. A few studies have demonstrated that high *Smad4* expression correlates to a high histological grade and advanced tumour stage in different types of cancer [41, 42]. Which target molecule *Smad4* is involved with can decide its function as an oncogenic or tumour suppressor. In groups with a negative correlation between *Smad4* and GATA3, *Smad4* has a greater influence in its tumour progression role along the GATA3-*Smad4*-fascin signalling pathway than its tumour suppression role. Additionally, the discrepancies might be also explained by several other factors, including study design (e.g., criteria of staining), type of malignancy, and ethnic factors. In the above studies showing a good prognosis, a positive expression of *Smad4* was defined as without considering only nuclear expression as the functional form of *Smad4*.

A prognostic model using a dual marker may be better than one using a single marker for patients with breast cancer. In the present study, the *Smad4*+/*GATA3*– status indicated a poor prognosis, whereas the *Smad4*–/*GATA3*+ status was associated with a good prognosis. In human breast cancer, these findings suggest that a high *Smad4* level resulting from low GATA3 expression can favour cancer progression according to fascin expression,

whereas a low *Smad4* level due to high GATA3 expression suppresses tumour cell proliferation and invasion according to fascin expression. In our study, fascin was highly expressed in the *Smad4*+/*GATA3*– group, compared with the *Smad4*–/*GATA3*+ group. In contrast to our expectations, our data did not show an inverse correlation between *Smad4* and GATA3; some cases with *Smad4*+/*GATA3*+ or *Smad4*–/*GATA3*– expression were observed in this study. In the negative correlation between *Smad4* and GATA3 through the GATA3-*Smad4*-fascin signalling pathway (*Smad4*–/*GATA3*+ vs. *Smad4*+/*GATA3*–), fascin expression was vastly different, which also influenced its survival rate. However, there can exist mechanisms that escape such an inverse correlation (*Smad4*+/*GATA3*+ or *Smad4*–/*GATA3*–), where the difference in survival rate is small, with a limited impact on fascin. Although it is difficult to understand the full mechanism, double positive (*Smad4*+/*GATA3*+) or double negative (*Smad4*–/*GATA3*–) can be influenced by signalling pathways that other unknown molecules are involved with. In other words, the functional correlation between *Smad4* and GATA3 may not apply in some portion of the TGF- β pathway and might depend on a range of environmental factors and/or different proteins linked to currently unknown mechanisms.

Our results revealed an alternative function of *Smad4* through the GATA3-*Smad4*-fascin signalling pathway in human breast cancer. Compared to its role as a tumour suppressor, the outcome of *Smad4* activity through this signalling pathway may be affected by the function of its target proteins according to the type of cancer, especially in breast cancer. Moreover, the combined expression pattern of *Smad4* and GATA3 was significantly associated with various clinicopathological parameters and patient survival. Therefore, understanding the relationship between *Smad4* and GATA3 may be helpful for predicting disease prognosis in patients with breast cancer.

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Statement of Ethics

This study (involving human participants) was approved by the Ethics Committee of the Kangbuk Samsung, Seoul, Republic of Korea (KBSMC 2015-07-035), and was performed according to the ethical standards of the Declaration of Helsinki, as revised in 2008.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: Global cancer statistics. *CA Cancer J Clin* 2011;61:69–90.
- Peppercorn J, Perou CM, Carey LA: Molecular subtypes in breast cancer evaluation and management: divide and conquer. *Cancer Invest* 2008;26:1–10.
- Naderi A, Teschendorff AE, Barbosa-Morais NL, Pinder SE, Green AR, Powe DG, Robertson JF, Aparicio S, Ellis IO, Brenton JD, Caldas C: A gene-expression signature to predict survival in breast cancer across independent data sets. *Oncogene* 2007;26:1507–1516.
- Jemal A, Center MM, DeSantis C, Ward EM: Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010;19:1893–1907.
- Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC Jr: American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol* 2007;25:5287–5312.
- Ahn SG, Jeong J, Hong S, Jung WH: Current issues and clinical evidence in tumor-infiltrating lymphocytes in breast cancer. *J Pathol Transl Med* 2015;49:355–363.
- Bombonati A, Sgroi DC: The molecular pathology of breast cancer progression. *J Pathol* 2011;223:307–317.
- Ali HR, Dawson SJ, Blows FM, Provenzano E, Leung S, Nielsen T, Pharoah PD, Caldas C: A Ki67/BCL2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. *J Pathol* 2012;226:97–107.
- Sun J, He H, Pillai S, Xiong Y, Challa S, Xu L, Chellappan S, Yang S: GATA3 transcription factor abrogates Smad4 transcription factor-mediated fascin overexpression, invadopodium formation, and breast cancer cell invasion. *J Biol Chem* 2013;288:36971–36982.
- Massague J: TGF β signalling in context. *Nat Rev Mol Cell Biol* 2012;13:616–630.
- Feldmann G, Beaty R, Hruban RH, Maitra A: Molecular genetics of pancreatic intraepithelial neoplasia. *J Hepatobiliary Pancreat Surg* 2007;14:224–232.
- Boulay JL, Mild G, Lowy A, Reuter J, Lagrange M, Terracciano L, Laffer U, Herrmann R, Rochlitz C: Smad4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer. *Br J Cancer* 2002;87:630–634.
- Dydensborg AB, Rose AA, Wilson BJ, Grote D, Paquet M, Giguere V, Siegel PM, Bouchard M: GATA3 inhibits breast cancer growth and pulmonary breast cancer metastasis. *Oncogene* 2009;28:2634–2642.
- Yoder BJ, Tso E, Skacel M, Pettay J, Tarr S, Budd T, Tubbs RR, Adams JC, Hicks DG: The expression of fascin, an actin-bundling motility protein, correlates with hormone receptor-negative breast cancer and a more aggressive clinical course. *Clin Cancer Res* 2005;11:186–192.
- Robbins P, Pinder S, de Klerk N, Dawkins H, Harvey J, Sterrett G, Ellis I, Elston C: Histological grading of breast carcinomas: a study of interobserver agreement. *Hum Pathol* 1995;26:873–879.
- Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch Pathol Lab Med* 2010;134:e48–e72.
- Rakha EA, Starczynski J, Lee AH, Ellis IO: The updated ASCO/CAP guideline recommendations for HER2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice. *Histopathology* 2014;64:609–615.
- Remmele W, Stegner HE: Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue (in German). *Pathologe* 1987;8:138–140.
- Masuda S: Breast cancer pathology: the impact of molecular taxonomy on morphological taxonomy. *Pathol Int* 2012;62:295–302.
- Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker J, Perou CM, Ellis MJ, Nielsen TO: Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst* 2009;101:736–750.
- Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, Langfort R, Waloszczyk P, Biernat W, Lasota J, Wang Z: GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2,500 epithelial and nonepithelial tumors. *Am J Surg Pathol* 2014;38:13–22.
- Heldin CH, Miyazono K, ten Dijke P: TGF- β signalling from cell membrane to nucleus through Smad proteins. *Nature* 1997;390:465–471.
- Kouros-Mehr H, Slorach EM, Sternlicht MD, Werb Z: GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. *Cell* 2006;127:1041–1055.
- Vignjevic D, Schoumacher M, Gavert N, Janssen KP, Jih G, Lae M, Louvard D, Ben-Ze'ev A, Robine S: Fascin, a novel target of β -catenin-TCF signaling, is expressed at the invasive front of human colon cancer. *Cancer Res* 2007;67:6844–6853.
- Min KW, Kim DH, Do SI, Chae SW, Kim K, Sohn JH, Pyo JS, Lee HJ, Kim DH, Oh S, Choi SH, Park YL, Park CH, Kim EK, Kwon MJ, Seo J, Moon KM: Negative association between GATA3 and fascin could predict relapse-free and overall survival in patients with breast cancer. *Virchows Arch* 2016;468:409–416.
- Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE: DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 1996;271:350–353.
- Wang LH, Kim SH, Lee JH, Choi YL, Kim YC, Park TS, Hong YC, Wu CF, Shin YK: Inactivation of SMAD4 tumor suppressor gene during gastric carcinoma progression. *Clin Cancer Res* 2007;13:102–110.
- Baldus SE, Schwarz E, Lohrey C, Zapotka M, Landsberg S, Hahn SA, Schmidt D, Dienes HP, Schmiegel WH, Schwarte-Waldhoff I: SMAD4 deficiency in cervical carcinoma cells. *Oncogene* 2005;24:810–819.
- Salovaara R, Roth S, Loukola A, Launonen V, Sistonen P, Avizienyte E, Kristo P, Jarvinen H, Souchelnytskyi S, Sarlomo-Rikala M, Aaltonen LA: Frequent loss of SMAD4/DPC4 protein in colorectal cancers. *Gut* 2002;51:56–59.
- Zhong D, Morikawa A, Guo L, Colpaert C, Xiong L, Nassar A, Chen C, Lamb N, Dong JT, Zhou W: Homozygous deletion of Smad4 in breast cancer cell lines and invasive ductal carcinomas. *Cancer Biol Ther* 2006;5:601–607.
- Sirard C, de la Pompa JL, Elia A, Itie A, Mirtos C, Cheung A, Hahn S, Wakeham A, Schwartz L, Kern SE, Rossant J, Mak TW: The tumor suppressor gene *Smad4/Dpc4* is required for gastrulation and later for anterior development of the mouse embryo. *Genes Dev* 1998;12:107–119.
- Tasca A, Stemig M, Broege A, Huang B, Davydova J, Zwijsen A, Umans L, Jensen ED, Gopalakrishnan R, Mansky KC: Smad1/5 and Smad4 expression are important for osteoclast differentiation. *J Cell Biochem* 2015;116:1350–1360.

- 33 Goto Y, Manabe N, Uchio-Yamada K, Yamaguchi-Yamada M, Inoue N, Yamamoto Y, Ogura A, Nagano N, Miyamoto H: Augmented cytoplasmic Smad4 induces acceleration of TGF- β 1 signaling in renal tubulointerstitial cells of hereditary nephrotic ICGN mice with chronic renal fibrosis; possible role for myofibroblastic differentiation. *Cell Tissue Res* 2004;315:209–221.
- 34 Tsuchida K, Zhu Y, Siva S, Dunn SR, Sharma K: Role of Smad4 on TGF- β -induced extracellular matrix stimulation in mesangial cells. *Kidney Int* 2003;63:2000–2009.
- 35 Xue J, Lin X, Chiu WT, Chen YH, Yu G, Liu M, Feng XH, Sawaya R, Medema RH, Hung MC, Huang S: Sustained activation of SMAD3/SMAD4 by FOXM1 promotes TGF- β -dependent cancer metastasis. *J Clin Invest* 2014;124:564–579.
- 36 Xie W, Mertens JC, Reiss DJ, Rimm DL, Camp RL, Haffty BG, Reiss M: Alterations of Smad signaling in human breast carcinoma are associated with poor outcome: a tissue microarray study. *Cancer Res* 2002;62:497–505.
- 37 Deckers M, van Dinther M, Buijs J, Que I, Lowik C, van der Pluijm G, ten Dijke P: The tumor suppressor Smad4 is required for transforming growth factor β -induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells. *Cancer Res* 2006;66:2202–2209.
- 38 Liu NN, Xi Y, Callaghan MU, Fribley A, Moore-Smith L, Zimmerman JW, Pasche B, Zeng Q, Li YL: Smad4 is a potential prognostic marker in human breast carcinomas. *Tumour Biol* 2014;35:641–650.
- 39 Liu N, Yu C, Shi Y, Jiang J, Liu Y: Smad4 expression in breast ductal carcinoma correlates with prognosis. *Oncol Lett* 2015;10:1709–1715.
- 40 Stuelten CH, Buck MB, Dippon J, Roberts AB, Fritz P, Knabbe C: Smad4-expression is decreased in breast cancer tissues: a retrospective study. *BMC Cancer* 2006;6:25.
- 41 Sheehan GM, Kallakury BV, Sheehan CE, Fisher HA, Kaufman RP Jr, Ross JS: Smad4 protein expression correlates with grade, stage, and DNA ploidy in prostatic adenocarcinomas. *Hum Pathol* 2005;36:1204–1209.
- 42 Hiwatashi K, Ueno S, Sakoda M, Kubo F, Tatenos T, Kurahara H, Mataka Y, Maemura K, Ishigami S, Shintchi H, Natsugoe S: Strong Smad4 expression correlates with poor prognosis after surgery in patients with hepatocellular carcinoma. *Ann Surg Oncol* 2009;16:3176–3182.