One DNA Methylation Regulates *CHIP* Gene Expression of Human Breast Cancer and Predicts Recurrence

TATSUYUKI GOHNO¹, TORU HANAMURA¹, MASAFUMI KUROSUMI²,
HIROYUKI TAKEI³, YURI YAMAGUCHI⁴ and SHIN-ICHI HAYASHI^{1,5}

¹Department of Molecular and Functional Dynamics,

Graduate School of Medicine, Tohoku University, Sendai, Japan;

²Department of Pathology, Saitama Cancer Center, Saitama, Japan;

³Division of Breast Surgery, Saitama Cancer Center, Saitama, Japan;

⁴Research Institute for Clinical Oncology, Saitama Cancer Center, Saitama, Japan;

⁵Center for Regulatory Epigenome and Disease, Graduate School of Medicine, Tohoku University, Sendai, Japan

Abstract. *Background/Aim:* Carboxyl terminus of Hsc70-interacting protein (CHIP) is a ubiquitin ligase that induces ubiquitination and degradation of its target proteins including oncoproteins. We reported that its down-regulation is associated with tumor progression and metastasis of breast cancer. However, the mechanism through which CHIP gene affects cancer cells is unclear. *Materials and Methods:* We extracted RNA from 45 primary breast cancer samples and compared CHIP mRNA expression profiles, promoter DNA methylation status, and clinicopathological information. *Results:* CHIP mRNA expression was significantly correlated with the tumor progression status. In several samples, a pinpoint CpG methylation in the CHIP gene promoter region was significantly correlated with CHIP mRNA expression. When this specific CpG was methylated in estrogen receptor (ER)-positive cases, a significant difference in 5-year recurrence was not found compared with ER-negative cases. *Conclusion:* CpG methylation contributes to the long-term prognosis of ER-positive breast cancer.

Recent studies have shown that the ubiquitin-proteasome pathway plays an important role in the regulation of tumor properties (1-3). This pathway controls selective degradation of short-lived proteins including many oncoproteins. Proteins

targeted for proteasomal degradation are sequentially tagged with ubiquitin by ubiquitinating enzymes E1, E2, and E3. In particular, E3 ubiquitin ligases play a pivotal role in ubiquitination. They catalyze the linkage of ubiquitin to the substrate protein, and polyubiquitinated substrates are degraded by the proteasome. E3 ubiquitin ligases have been studied extensively in the field of cancer, and some of them have emerged as therapeutic targets.

Carboxyl terminus of heat shock cognate (Hsc) 70-interacting protein (CHIP) is a U-box-type E3 ubiquitin ligase (4, 5). CHIP interacts with Hsc70/90 and is involved in ubiquitination and degradation of its target proteins including several oncoproteins (6-8). In breast cancer, CHIP plays a key role in degradation of misfolded estrogen receptor alpha (ER α) and human epidermal growth factor receptor 2 (HER2) (9-11). Recently, we reported that down-regulation of CHIP is significantly associated with tumor progression and metastasis of human breast cancer (12). We found that CHIP expression levels are markedly down-regulated in advanced tumor samples, such as node metastasis-positive and late-stage cancers, compared with normal samples. Thus, CHIP is closely associated with the malignancy of breast cancer, but the mechanism that regulates its mRNA expression is unclear. To elucidate this mechanism, we focused on a representative gene regulatory mechanism, DNA methylation of the gene promoter region. It is well known that epigenetic regulation such as DNA methylation plays crucial roles in mRNA expression (13-15). Aberrant DNA methylation of CpG islands in gene promoter regions leads to silencing of several important genes including tumor suppressor genes, and the CHIP gene has a CpG island in its promoter region. Therefore, in this study, we analyzed the DNA methylation status of the CHIP gene promoter region and compared it with its mRNA expression levels among 45 breast tumor samples. Moreover, to confirm

This article is freely accessible online.

Correspondence to: Tatsuyuki Gohno, Ph.D., Department of Molecular and Functional Dynamics, Graduate School of Medicine, Tohoku University, 2-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, Japan. Tel: +81 227177472, Fax: +81 227177475, e-mail: gohno717@gmail.com

Key Words: Breast cancer recurrence, CHIP, DNA methylation, ER.

Table I. Relationship between CHIP mRNA expression and clinicopathological factors.

Characteristic	n	CHIP mRNA	p-Value	Characteristic	n	CHIP mRNA	p-Value
Age				Stage			
<50	12	1.77±1.45	0.2085	I	2	2.91±0.76	0.0295
≥50	33	1.27±1.44		II	28	1.75±1.27	
ER				III	9	0.69±1.35	
Positive	25	1.57±1.32	0.5526	IV	6	0.38±1.72	
Negative	20	1.21±1.60		T			
PgR				1	4	2.86±0.45	0.0614
Positive	21	1.56±1.37	0.5851	2	29	1.46±1.37	
Negative	24	1.28±1.52		3	6	0.74±1.17	
Node Metastasis				4	6	0.86±1.95	
Positive	20	0.81±1.54	0.0165	N			
Negative	25	1.89±1.19		0	8	2.11±0.95	0.3065
Tumor Size (mm)				1	29	1.35±1.55	
<25	17	1.69±1.19	0.468	2	5	0.56±1.30	
≥25	28	1.23±1.58		3	3	1.49±1.45	
				M			
				0	39	1.56±1.36	0.0950
				1	6	0.38±1.72	

Significant *p*-Values are shown in bold.

whether the DNA methylation status is associated with clinicopathological characteristics and prognosis, we compared the methylation status with the clinicopathological characteristics and recurrence rate of patients.

Materials and Methods

Tumor samples. Primary human breast cancer tissues were obtained during surgery from 45 patients after patient consent from 1999 to 2001 at Saitama Cancer Center Hospital (Saitama, Japan), embedded in optimal cutting temperature (OCT), and transferred to Tohoku University. The study was approved by Tohoku University Ethics Committee (Approval No. 2009-81). The estrogen receptor (ER) and progesterone receptor (PgR) protein expression in the samples were determined by enzyme immunoassays. The cut off value for ER and PgR was set at 10 units.

RNA preparation and real-time RT-PCR. Several 5 µm-thick sections were prepared from tumor tissues embedded in OCT and mounted on film-coated glass slides. Then, each section was fixed, dehydrated with methanol, and stained with toluidine blue staining prior to laser capture microdissection (LCM). The sections were also stained with hematoxylin-eosin to determine the outlines of tumor areas. Only outlined tumor areas were microdissected using a Leica LMD7000 (Leica Microsystems GmbH, Wetzlar, Germany). RNA was extracted from the collected tumor cells using an RNeasy Micro Kit (Qiagen, Mississauga, Canada), according to the manufacturer's instructions. Total RNA was converted to first-strand cDNA using a QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was performed using a StepOne™ Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA). The oligonucleotides used in real-time

PCR were as follows: forward primer 5'-GGA GAG CTA TGA TGA GGC CAT C-3', reverse primer 5'-AAG TTC AGC CGC TGC TCC T-3', and probe 5'-CTG CAG CGA GCT TAC AGC CTG GC-3' for CHIP; forward primer 5'-GAG TAC TTG CGC TCA GGA GGA-3', reverse primer 5'-ACG TGG ACA TCC GCA AAG A-3', and probe 5'-CAA TGA TCT TGA TCT TCA TTG TGC TGG GTG-3' for β-actin as the internal control.

DNA preparation and bisulfite sequencing. Several 40 µm-thick sections containing a large tumor fragment were prepared from tissues embedded in OCT. DNA from these tumor samples was extracted using a QIAamp DNA Mini Kit (Qiagen), according to the manufacturer's instructions. Extracted DNA was treated with bisulfite using an EpiTect Bisulfite Kit (Qiagen), according to the manufacturer's instructions. Then, nested PCR was carried out at 95°C for 2 min, followed by 42 cycles (40 cycles for 2nd PCR) at 95°C for 30 s, 48°C for 30 s (1 min for 2nd PCR), 72°C for 1 min, and final extension at 72°C for 7 min. The oligonucleotides used in nested PCR were as follows: 1st PCR forward primer 5'-TTG GAT TTA TTA GGG AGG TT-3', 2nd PCR forward primer 5'-TTA AGT TGT TAG GTT AGT AG-3', and common reverse primer 5'-CTA AAC TAC CAT TCT AAA AC-3'. The PCR amplicon was electrophoresed, and DNA from the correctly sized band was extracted using a QIAquick Gel Extraction Kit (Qiagen). The purified DNA was sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems Inc.).

Statistical analysis. Statistical analysis was performed with the StatFlex 6.0 software program (Artech Co., Ltd., Osaka, Japan). Comparison of two independent groups was performed with the Mann-Whitney *U*-test. For comparison among three groups or more, the Kruskal-Wallis test was used. *p*<0.05 was considered to be statistically significant.

Figure 1. Analyzed region in the *CHIP* gene promoter. The region analyzed by bisulfite DNA sequencing was 249 bp from -600 bp to -352 bp of the transcription initiation site in the *CHIP* gene. The horizontal arrow indicates the sequence of the analyzed region, and the curved arrow indicates the transcription initiation site. The gray area indicates a CpG island.

Results

CHIP mRNA expression significantly correlates with tumor progression of breast cancer tissue. To examine the role of *CHIP* gene in breast cancer, we assessed the relationship between the *CHIP* mRNA expression and clinicopathological characteristics of the cases (Table I). *CHIP* mRNA was significantly decreased with lymph node metastasis and stage progression. Other factors related with tumor progression such as the tumor size and TNM status tended to be associated with lower *CHIP* mRNA expression although without statistical significance. The *CHIP* gene is an ER target with an ERE half site in its promoter region, but *CHIP* mRNA expression was not significantly correlated with expression of ER protein or its target gene PgR. These results suggest that another transcription activation mechanism regulates the expression of *CHIP* gene in addition to ER-dependent transcription.

Promoter methylation of the CHIP gene regulates its expression. We hypothesized that down-regulation of *CHIP* expression in breast cancer occurred through promoter methylation as a representative epigenetic regulation system. Therefore, bisulfite-specific PCR was performed using DNA extracted from the samples, and the DNA methylation status was determined by analysis of the sequence of the PCR product. The analyzed region was 249 bp from -600 bp to -352 bp of the transcription initiation site in the *CHIP* gene (Figure 1). This region was identified as a domain containing the region closely related to transcriptional activation using a luciferase reporter assay in preliminary experiments (data not shown). Results from the methylation analysis of the 38 breast cancer tissues indicated that methylation occurred in the 4th to 9th CpG sites of the region in 50% (19/38) of the

samples (Figure 2). Samples with no less than 50% (three point) methylation of these six CpG sites were designated as positive for methylation. Using this threshold, samples were divided into methylation (M) and unmethylation (U) groups. We then compared the methylation status of M and U groups with the *CHIP* mRNA expression status (Figure 3A). Although there was no statistical significance, the methylation status seemed to correlate with *CHIP* mRNA expression. Interestingly, there were six samples with only the 9th CpG (CpG9) unmethylated in the M group, whereas one sample only had CpG9 methylated in the U group (Figure 2). Therefore, we divided the samples into CpG9 M and CpG9 U groups and compared the CpG9 methylation status with the *CHIP* mRNA expression status (Figure 3B). Statistical analysis revealed significant intergroup differences, namely *CHIP* mRNA expression was significantly decreased in the CpG9 M group compared with the CpG9 U group. However, no significant difference in *CHIP* mRNA expression was observed between groups with other CpG pinpoint methylations (data not shown).

DNA methylation coordinately regulates CHIP mRNA expression with ER. As shown in Table I, the ER status was not correlated with *CHIP* mRNA expression, even though *CHIP* is an ER target gene. It was assumed that this pinpoint CpG9 methylation interfered with the mechanism through which ER regulates *CHIP* mRNA expression. Therefore, ER-positive and -negative groups were classified into M and U groups, and *CHIP* mRNA expression of the four groups was compared (Figure 4). Interestingly, remarkable down-regulation of *CHIP* mRNA expression was observed only in the ER-negative M group. In contrast, the other three groups maintained high mRNA expression, especially the ER-negative U group ($p < 0.05$). These results suggest that *CHIP* gene is

Figure 2. DNA methylation status of the CHIP gene in breast tumor tissues. (Left) The region analyzed by bisulfite DNA sequencing included 17 CpG sites. White circles indicate unmethylated CpG sites, and black circles indicate methylated CpG sites. Samples with no less than 50% (three point) methylation in the 4th to 9th CpG sites were designated as the region-methylated group. (Right) CHIP mRNA expression levels of four groups divided by region methylation and the CpG9 methylation status. Black bars are the region-methylated/CpG9-methylated group. Gray bars are the region-methylated/CpG9-unmethylated group. Light gray bars are the region-unmethylated/CpG9-methylated group. White bars are the region-unmethylated/CpG9-unmethylated group.

complimentarily regulated by ER and DNA methylation. Specifically, the reason that there was no difference in ER-positive and -negative groups was CpG methylation.

CpG9 methylation of the CHIP gene predicts long term recurrence. CHIP expression suppresses distal metastasis (12). Therefore, we investigated whether CpG9 methylation-regulated expression was associated with tumor recurrence

after 5 years. Although the number of patients with recurrence in the ER-positive group was significantly lower than that in the ER-negative group, some metastatic recurrence was observed in patients despite ER positivity (Figure 5A). In the analysis of the four groups divided by ER positivity and CpG9 methylation (Figure 5B), the recurrence rate in the ER-positive/GpG9 U group was significantly lower than that in the two ER-negative groups.

Figure 3. *CHIP* mRNA expression level classified by the methylation status in patients. The intergroup difference of *CHIP* mRNA expression in patients divided into methylated or unmethylated groups is shown. Box plots show the intergroup difference of the (A) CpG4-9 and (B) CpG9 methylation status in each group.

However, in the ER-positive/CpG9 M group, there was no significant difference in the recurrence rate compared with the two ER-negative groups. These results suggest that ER-positive breast cancer can be divided in two prognostic groups by the CpG9 methylation status. Thus, ER and methylation status may be valuable for prognosis prediction.

Discussion

When determining a therapeutic strategy for breast cancer, the resistance mechanism is one of the most important considerations. ER is the factor that relates most strongly to breast cancer proliferation and progression, and is used to predict the efficacy of hormonal therapy (16-20). Generally, although ER-positive breast cancer is considered to have a good prognosis compared with ER-negative breast cancer, it is not necessarily an indicator of long-term recurrence. According to the U.S. patient registration database in 1992-2007 (21), ER-negative cases have a peak recurrence rate at an early stage compared to 1-3 years postoperatively, and the rate decreases over time. However, ER-positive cases maintain a constant rate of recurrence in the long term,

Figure 4. *CHIP* mRNA expression level classified by the methylation status and ERα in patients. The intergroup difference of *CHIP* mRNA expression in patients divided into methylated or unmethylated and ER-positive or -negative groups is shown. Box plots show the intergroup difference of the CpG9 methylation status in each group.

Figure 5. Recurrence rate after 5 years in patients divided by ERα expression and CpG9 methylation. The intergroup difference of the recurrence rate after 5 years in patients divided into ER positive/negative and CPG9-methylated/unmethylated groups is shown. Bars indicate the intergroup difference of (A) only ERα, and (B) ERα and CpG9 methylation in each group. Gray bars indicate the non-recurrence group, and black bars indicate the recurrence group. * $p < 0.05$, †N.D.

which is higher than the rate of ER-negative cases at 5 years postoperatively. According to reports of the recurrence risk in many countries, the recurrence risk of ER-positive breast cancer exceeds that of ER-negative breast cancer at 3-4 years postoperatively (21). It is necessary to follow-up patients

ER-positive breast cancer for several years postoperatively for recurrence. Therefore, new additional indices are required for ER-positive breast cancer.

CHIP is an ER target gene with an important role in breast cancer (4-12). *CHIP* mRNA expression in clinical samples was negatively correlated with tumor progression, especially lymph node metastasis and advanced stage. We have reported that *CHIP* promotes degradation of SRC-3 (12), an important coactivator of ER (22-25), as a target of ubiquitination in breast cancer. SRC-3 elevates the transcriptional activity of Smad2 and expression of Twist1, and causes tumor metastasis (12, 26). Additionally, SRC-3 causes resistance to endocrine therapy in primary breast cancer by targeting phosphorylation pathways such as HER2 (27, 28). Similar to previous reports, in our samples, SRC-3 degradation may have been associated with repression of tumor progression and metastasis. It has been reported that *CHIP* expression leads to regression of cancer. Although *CHIP* is a target gene of ER, its expression was not correlated with ER expression in previous reports or this study. Thus, there may be mechanisms regulating its expression other than those mediated by ER.

To delineate the expression mechanism, we focused on the epigenetic regulation of the *CHIP* gene and analyzed the DNA methylation status of its promoter region in clinical samples. Methylation was identified in the 4th to 9th CpG sites in 50% of the samples. Furthermore, methylation of the 9th CpG in this region was significantly correlated with down-regulation of *CHIP* mRNA expression. Analysis of transcription factor binding sites in this domain revealed binding of c-Ets, c-Myb, and c-Rel, which are methylation-sensitive transcription factors. If CpG islands are methylated, binding of these transcriptional factors is inhibited by the methyl group itself or a methyl CpG-binding protein, and transcriptional activity is down-regulated (29, 30). Therefore, methylation transcription factor recognition sequence in this region may be a mechanism through which *CHIP* expression is regulated.

CHIP mRNA expression did not correlate with expression of ER protein, a known *CHIP* genetic control factor (12). To investigate the mechanism that regulates transcription of the *CHIP* gene independently of ER, the samples were divided into four groups by positivity for the ER and CpG9 methylation. High expression of *CHIP* mRNA was observed in the CpG9 U group regardless of ER expression. Moreover, in the CpG9 M group, down-regulation of *CHIP* mRNA was observed only in the ER-negative group. Because *CHIP* mRNA was highly expressed except in the ER-negative/CpG9 M group, *CHIP* gene expression may be regulated by two mechanisms employing ER and/or CpG9 methylation. Furthermore, methylation of CpG9 and its nearby region was significantly enhanced in a sample from a <50 years old (data not shown). This result suggests that the down-regulation by DNA methylation occurs in a young

population, and that the regulation mechanism involves malignancies such as juvenile breast cancer.

We also analyzed the tumor recurrence rate. ER is the one of the most valuable prognostic factors of breast cancer (16-20). Our analysis also reflected this tendency. The ER-positive group showed a significantly lower recurrence rate than the ER-negative group. However, in the ER-positive group, there were some patients with cancer recurrence. Even in ER-positive cases, the CpG9-methylated group showed no significant difference in recurrence rate compared to each of the ER-negative methylation groups. Previously, we reported the relationship between ER and *CHIP* protein expression (31), and recurrence-free survival was significantly better in the ER-positive cases with high *CHIP* expression than in ER-negative cases. However, although positive for ER, such cases with low *CHIP* expression tended to have worse recurrence-free survival than those with high *CHIP* expression. Additionally, there are several reports of expression regulated by pinpoint CpG methylation in breast cancer (32, 33). Our results and these reports suggest that this methylation of CpG9 can also predict the long-term prognosis of ER-positive breast cancer.

It is difficult to specify the prognosis of many patients by only one or a few factors such as protein expression. Additional factors that increase the accuracy of prognosis prediction in combination with existing factors should be identified. Epigenetic modifications such as DNA methylation or histone modification are powerful predictive factors and should be further investigated.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

Tatsuyuki Gohno: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft; Toru Hanamura: Methodology, Investigation, Writing - review and editing; Masafumi Kurosumi: Methodology, Investigation, Writing - review and editing; Hiroyuki Takei: Methodology, Investigation, Writing - review and editing; Yuri Yamaguchi: Methodology, Investigation, Writing - review and editing; Shin-Ichi Hayashi: Conceptualization, Methodology, Investigation, Writing - review and editing, Supervision, project administration, funding acquisition.

Acknowledgements

The Authors thank Toshifumi Niwa, Yuko Seino and Chika Tazawa for valuable assistance and comments on this study. The Authors also thank M. Arico from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan, a Grant-in-Aid for

Cancer Research from the Ministry of Health, Labor, and Welfare, Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), and a Grant from the Smoking Research Foundation.

References

- Hochstrasser M: Ubiquitin-dependent protein degradation. *Annu Rev Genet* 30: 405-439, 1996. PMID: 8982460. DOI: 10.1146/annurev.genet.30.1.405
- Hershko A and Ciechanover A: The ubiquitin system. *Annu Rev Biochem* 67: 425-479, 1998. PMID: 9759494. DOI: 10.1146/annurev.biochem.67.1.425
- Konstantinova IM, Tsimokha AS and Mittenberg AG: Role of proteasomes in cellular regulation. *Int Rev Cell Mol Biol* 267: 59-124, 2008. PMID: 18544497. DOI: 10.1016/S1937-6448(08)00602-3
- Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY and Patterson C: Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* 19(6): 4535-4545, 1999. PMID: 10330192. DOI: 10.1128/MCB.19.6.4535
- Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Höhfeld J and Patterson C: The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat Cell Biol* 3(1): 93-96, 2001. PMID: 11146632. DOI: 10.1038/35050618
- Meacham GC, Patterson C, Zhang W, Younger JM and Cyr DM: The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat Cell Biol* 3(1): 100-105, 2001. PMID: 11146634. DOI: 10.1038/35050509
- Esser C, Scheffner M and Höhfeld J: The chaperone-associated ubiquitin ligase CHIP is able to target p53 for proteasomal degradation. *J Biol Chem* 280(29): 27443-27448, 2005. PMID: 15911628. DOI: 10.1074/jbc.M501574200
- Li D, Marchenko ND, Schulz R, Fischer V, Velasco-Hernandez T, Talos F and Moll UM: Functional inactivation of endogenous MDM2 and CHIP by HSP90 causes aberrant stabilization of mutant p53 in human cancer cells. *Mol Cancer Res* 9(5): 577-588, 2011. PMID: 21478269. DOI: 10.1158/1541-7786.MCR-10-0534
- Tateishi Y, Kawabe Y, Chiba T, Murata S, Ichikawa K, Murayama A, Tanaka K, Baba T, Kato S and Yanagisawa J: Ligand-dependent switching of ubiquitin-proteasome pathways for estrogen receptor. *EMBO J* 23(24): 4813-4823, 2004. PMID: 15538384. DOI: 10.1038/sj.emboj.7600472
- Fan M, Park A and Nephew KP: CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor- α . *Mol Endocrinol* 19(12): 2901-2914, 2005. PMID: 16037132. DOI: 10.1210/me.2005-0111
- Xu W, Marcu M, Yuan X, Mimnaugh E, Patterson C and Neckers L: Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB2/Neu. *Proc Natl Acad Sci USA* 99(20): 12847-12852, 2002. PMID: 12239347. DOI: 10.1073/pnas.202365899
- Kajiro M, Hirota R, Nakajima Y, Kawanowa K, So-ma K, Ito I, Yamaguchi Y, Ohie SH, Kobayashi Y, Seino Y, Kawano M, Kawabe Y, Takei H, Hayashi S, Kurosumi M, Murayama A, Kimura K and Yanagisawa J: The ubiquitin ligase CHIP acts as an upstream regulator of oncogenic pathways. *Nat Cell Biol* 11(3): 312-319, 2009. PMID: 19198599. DOI: 10.1038/ncb1839
- Bird A: DNA methylation patterns and epigenetic memory. *Genes Dev* 16(1): 6-21, 2002. PMID: 11782440. DOI: 10.1101/gad.947102
- Reik W: Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 447(7143): 425-432, 2007. PMID: 17522676. DOI: 10.1038/nature05918
- Esteller M: Epigenetics in cancer. *N Engl J Med* 358(11): 1148-1159, 2008. PMID: 18337604. DOI: 10.1056/NEJMra072067
- 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 and Wolff AC: American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 28(16): 2784-2795, 2010. PMID: 20404251. DOI: 10.1200/JCO.2009.25.6529
- Burstein HJ, Prestrud AA, Seidenfeld J, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Malin J, Mamounas EP, Rowden D, Solky AJ, Sowers MR, Stearns V, Winer EP, Somerfield MR, Griggs JJ and American Society of Clinical Oncology: American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol* 28(23): 3784-3796, 2010. PMID: 20625130. DOI: 10.1200/JCO.2009.26.3756
- Pujol P, Daures JP, Thezenas S, Guilleux F, Rouanet P and Grenier J: Changing estrogen and progesterone receptor patterns in breast carcinoma during the menstrual cycle and menopause. *Cancer* 83(4): 698-705, 1998. PMID: 9708933.
- Yashiki SI, Eguchi H, Tanimoto K, Yoshida T, Omoto Y, Inoue A, Yoshida N and Yamaguchi Y: The expression and function of estrogen receptor α and β in human breast cancer and its clinical application. *Endocr Relat Cancer* 10(2): 193-202, 2003. PMID: 12790782. DOI: 10.1677/erc.0.0100193
- Hayashi S, Niwa T and Yamaguchi Y: Estrogen signaling pathway and its imaging in human breast cancer. *Cancer Sci* 100(10): 1773-1778, 2009. PMID: 19575750. DOI: 10.1111/j.1349-7006.2009.01243.x
- Lin NU and Winer EP: Advances in adjuvant endocrine therapy for postmenopausal women. *J Clin Oncol* 26(5): 798-805, 2008. PMID: 18258989. DOI: 10.1200/JCO.2007.15.0946
- Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM and Meltzer PS: AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* 277(5328): 965-968, 1997. PMID: 9252329. DOI: 10.1126/science.277.5328.965
- Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y and Evans RM: Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* 90(3): 569-580, 1997. PMID: 9267036. DOI: 10.1016/s0092-8674(00)80516-4
- Takeshita A, Cardona GR, Koibuchi N, Suen CS and Chin WW: TRAM-1, A novel 160-kDa thyroid hormone receptor activator molecule, exhibits distinct properties from steroid receptor coactivator-1. *J Biol Chem* 272(44): 27629-27634, 1997. PMID: 9346901. DOI: 10.1074/jbc.272.44.27629

- 25 Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK and Rosenfeld MG: The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* 387(6634): 677-684, 1997. PMID: 9192892. DOI: 10.1038/42652
- 26 Thuault S, Valcourt U, Petersen M, Manfioletti G, Heldin CH and Moustakas A: Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition. *J Cell Biol* 174(2): 175-183, 2006. PMID: 16831886. DOI: 10.1083/jcb.200512110
- 27 Bouras T, Southey MC and Venter DJ: Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/neu. *Cancer Res* 61(3): 903-907, 2001. PMID: 11221879.
- 28 Shou J, Massarweh S, Osborne CK, Wakeling AE, Ali S, Weiss H and Schiff R: Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J Natl Cancer Inst* 96(12): 926-935, 2004. PMID: 15199112. DOI: 10.1093/jnci/djh166
- 29 Clouaire T and Stancheva I: Methyl-CpG binding proteins: specialized transcriptional repressors or structural components of chromatin? *Cell Mol Life Sci* 65(10): 1509-1522, 2008. PMID: 18322651. DOI: 10.1007/s00018-008-7324-y
- 30 Ooi SK and Bestor TH: The colorful history of active DNA demethylation. *Cell* 133(7): 1145-1148, 2008. PMID: 18585349. DOI: 10.1016/j.cell.2008.06.009
- 31 Kurozumi S, Yamaguchi Y, Hayashi S, Hiyoshi H, Suda T, Gohno T, Matsumoto H, Takei H, Horiguchi J, Takeyoshi I, Oyama T and Kurosumi M: Prognostic value of the ubiquitin ligase carboxyl terminus of the Hsc70-interacting protein in postmenopausal breast cancer. *Cancer Med* 5(8): 1873-1882, 2016. PMID: 27334118. DOI: 10.1002/cam4.780
- 32 Tsuboi K, Nagatomo T, Gohno T, Higuchi T, Sasaki S, Fujiki N, Kurosumi M, Takei H, Yamaguchi Y, Niwa T and Hayashi SI: Single CpG site methylation controls estrogen receptor gene transcription and correlates with hormone therapy resistance. *J Steroid Biochem Mol Biol* 171: 209-217, 2017. PMID: 28412323. DOI: 10.1016/j.jsbmb.2017.04.001
- 33 Fürst RW, Kliem H, Meyer HH and Ulbrich SE: A differentially methylated single CpG-site is correlated with estrogen receptor alpha transcription. *J Steroid Biochem Mol Biol* 130(1-2): 96-104, 2012. PMID: 22342840. DOI: 10.1016/j.jsbmb.2012.01.009

Received November 25, 2021

Revised December 13, 2021

Accepted December 14, 2021