The Korean Journal of Pathology 2009; 43: 400-7
DOI: 10.4132/KoreanJPathol.2009.43.5.400

The Loss of E-cadherin is Associated with the Epigenetic Alteration of *CDH1* in Breast Cancer and it is also Associated with an Abnormal β-catenin Expression in Lobular Carcinoma

Gwangil Kim · Ji Young Kim[1]
Hee Jung An · Haeyoun Kang
Tae Heon Kim · Jung Yon Shim[1]
Jin Hyung Heo · Hai Lin Park[2]
Young Kil Choi[3]

Department of Pathology, Bundang CHA Medical Center, School of Medicine, CHA University, Seongnam; Departments of [1]Pathology, [2]Surgery, and [3]Internal Medicine, Gangnam CHA Medical Center, School of Medicine, CHA University, Seoul, Korea

Received : September 11, 2008
Accepted : June 2, 2009

Corresponding Author
Gwangil Kim, M.D.
Department of Pathology, Bundang CHA Medical Center, 351 Yatap-dong, Bundang-gu, Seongnam 463-712, Korea
Tel: 031-780-5452
Fax: 031-780-5476
E-mail: blacknw@hanmail.net

Background : APC and E-cadherin are the key molecules in the Wnt/β-catenin pathway. We attempted to define the epigenetic alteration of *APC* and *CDH1* (the E-cadherin gene) and the expression of Wnt-related molecules in human mammary carcinomas. **Methods :** Sixty-four mammary carcinomas, including 52 invasive ductal carcinomas (IDCs) and 12 invasive lobular carcinomas (ILCs), were evaluated using methylation-specific PCR and immunohistochemistry. We performed immunohistochemistry for E-cadherin, β-catenin, APC, Wnt1, cyclin D1, ER, PR and C-erb B2. **Results :** Hypermethylation of *APC* and *CDH1* was observed in 38 (59%) and 28 (44%) cases, respectively. *CDH1* hypermethylation in ILCs was increased compared to that in IDCs (p=0.002) and it was associated with the loss of E-cadherin (p=0.02) and β-catenin (p=0.042). *APC* methylation was positively correlated with the ER expression (p=0.021). Abnormal cytoplasmic localization of β-catenin was found in 10 cases and any expression was not detected in six cases. In ILCs, the E-cadherin or β-catenin expression was markedly decreased compared to that in IDCs (p<0.001 in both). **Conclusions :** Methylation of *APC* or *CDH1* was relatively frequent in mammary carcinomas. The loss of E-cadherin in mammary carcinoma was associated with *CDH1* methylation, and abnormal β-catenin expression was related to the loss of E-cadherin in ILC.

Key Words : Breast neoplasms; Wnt1 protein; Beta catenin; DNA methylation; E-cadherin

Breast cancer is the second most common cause of cancer death for females in the world. In the Republic of Korea, it has been the most common malignancy in adult women since 2001.[1] Although many previous studies of these tumors have been performed, the molecular carcinogenesis of breast cancer has not yet been fully determined.

Wnt signaling is known to contribute to the development of various malignant tumors, and especially gastrointestinal malignancies such as colon cancer, and Wnt signaling is also known to regulate stem cell development. APC and E-cadherin are key tumor suppressor molecules in a variety of cancers and they also play central role in the canonical or non-canonical Wnt pathway. In the canonical pathway, a defective APC-GSK3 β-axin-β-catenin complex induces an abnormal nuclear/cytoplasmic localization of the β-catenin and this activates a transcription factor that accelerates cell proliferation. APC defects are known to be highly correlated with the destruction of this catenin complex.[2,3] Although somatic mutation or the loss of heterozygosity (LOH) of *APC* and *CDH1* (E-cadherin gene) is frequently seen in gastrointestinal carcinomas, there have been only a few reported mutations of *APC* in mammary tumors.[4]

E-cadherin is a representative adhesion molecule of the epithelial cells; it comprises a membranous structural complex with β-catenin, and this complex functions in cell-cell identification and tissue morphogenesis. When carcinoma cells lose E-cadherin, they have a chance to become poorly differentiated tumor cells and gain an infiltrative character, and they can ultimately become capable of metastasizing.[5]

Mammary lobular carcinomas have characteristic clinico-pathological features compared with the ductal carcinomas. The tumor cells of mammary lobular carcinomas are relatively small and they lack cellular adhesion with little tubule formation. Because ductal carcinomas do not make tubules in many cases, the histological features cannot perfectly discriminate between these two types of lesions. When performing immunohistochemical staining, E-cadherin is completely lost in almost all the mammary lobular carcinoma cells and this is in contrast to the ductal carcinomas, in which no change or only a slight decrease of staining intensity is observed in 80-100%.[6,7] The loss of E-cadherin is not always caused by the gene defect itself.[8] Hypermethylation of the gene promoter CpG islands is associated with delayed replication, condensed chromatin and transcriptional inhibition.[9] Therefore, the transcriptional suppression mechanism of gene promoter hypermethylation of *APC* and *CDH1* has been examined in two representative histological mammary carcinoma types (ductal and lobular carcinomas), respectively.[10-12]

Of the 19 Wnt genes, *Wnt1* was the first to be proven to be a mammary oncogene in mammary tumor virus (MMTV)-induced tumors.[13] The expression of its human homolog transcript in human breast cancer tissue has rarely been described.[14,15]

In this study, we examined and compared the methylation status of the *APC* and *CDH1* promoter CpG islands in human mammary ductal and lobular carcinomas. We also investigated the expression profiles of the Wnt pathway-related molecules and Wnt1. We were able to discover the relationship between the promoter CpG island hypermethylation statuses of *APC* and *CDH1* and the expression of Wnt-related molecules, including β-catenin, in human mammary carcinomas.

MATERIALS AND METHODS

Case selection

We analyzed 64 formalin-fixed, paraffin-embedded tissues of sporadic mammary carcinomas that were surgically resected between January 2001 and December 2004. They were collected from the archives of the Department of Pathology, CHA General Hospital, CHA University with Institutional Review Board approval. All the patients were female with a mean age of 49 years (range: 29-81 years). Fifty-two invasive ductal carcinomas (IDC) and 12 invasive lobular carcinomas (ILC) were included. All tumors were newly diagnosed, and the recurrent or hereditary lesions were excluded. Of the 64 invasive cancers, 23 cases were in stage I, 27 were in stage II and 14 were in stage III (Table 1).

DNA extraction

Manual microdissection on H&E stained slides for DNA extraction was performed on the formalin-fixed, paraffin-embedded specimens by using a 27½-gauge injection needle under a low-power ($\times$4) objective view. The tumor areas with more than 90% cellularity were chosen for excision. The paired normal tissue was also obtained and analyzed to exclude germ line mutations. The DNA was extracted by using a previously described method.[16]

Bisulfite modification of the DNA and methylation-specific polymerase chain reaction (MSP)

The method for determining the status of the CpG island methylation of *APC* and *CDH1* was performed as described previously.[17] The DNA was denatured with 2 mol/L NaOH at 37℃ for 10 min, and this was followed by incubation with 3 mol/L sodium bisulfite (pH 5.0) at 50℃ for 16 h in the dark. After treatment, the DNA was purified using the DNA purification kit (Promega, Madison, WI, USA) as recommended by the manufacturer; it was incubated with 3 mol/L NaOH at room temperature for 20 min and then it was precipitated with ethanol. The methylation status of each gene was determined using 2 μL of modified DNA as a template for the PCR with using primers that are specific for the methylated and unmethylated alleles. The previously reported primers for the unmethylated or methylated sequence of *APC* gene promoter 1A and *CDH-1* were used.[18,19] The sequences for *APC* were as follows: U-f: 5′-GTGTTTTATTGTGGAGTGTGGGTT-3′, U-r 5′;-CCAATCAACAAACTCCCAACAA-3′, M-f 5′-TATTGCGGAGTGCGGGTC-3′, and M-r 5′-TCGACGAACTCCCGACGA-3′. The sequences for CDH1 were as follows: U-f 5′-TAATTTTAGGTTAGAGGGTTATTGT-3′, U-r 5′-CACAACCAATCAACAACACA-3′, M-f 5′-TTAGGTTAGAGGGTTATCGCGT-3′, and M-r 5′-TAACTAAAAATTCACCTACCGAC-3′. PCR was performed for 35 cycles at 95℃ for denaturing, 55.5℃ for annealing (53.5℃ for *CDH1*), and 72℃ for extension with a final extension step of 5 min. Seven μL of the PCR products were electrophoresed on 2% agarose gels and they were visualized by ethidium bromide staining. Commercially manufactured methylated and unmethylated DNAs (CpGenome Universal Methylated and Unmethylated DNA, Chemicon, CA, USA) were used as controls. The samples showing signals approximately equiva-

Table 1. Correlation of the clinicopathological characteristics or immunohistochemical result of the patients and the results of methylation status of APC and E-cadherin genes in the invasive mammary carcinomas

Clinicopathological information		No. of cases	Methylation markers					
			APC			*CDH1*		
			M	U	p-value*	M	U	p-value*
Age (years)					**0.045**			0.667
	≤50	39	27	12		16	23	
	>50	25	11	14		12	13	
Diagnosis					0.225			**0.002**
	IDC	52	29	23		18	34	
	ILC	12	9	3		10	2	
Histological grade (IDC, n=52)					0.332			0.29
	I	22	13	9		5	17	
	II	15	6	9		6	9	
	III	15	10	5		7	8	
T stage					0.082			**<0.001**
	T1	31	15	16		10	21	
	T2	33	23	10		18	5	
N stage					0.72			0.147
	N0-1	51	29	22		20	31	
	N2-3	13	9	4		8	5	
Clinical stage					0.520			0.32
	I	23	11	12		10	13	
	II	27	17	10		9	18	
	III	14	10	4		9	5	
E-cadherin					0.957			**0.042**
	Positive	47	28	19		17	30	
	Negative	17	10	7		11	6	
β-catenin					0.398			**0.02**
	Membranous	48	27	27		17	31	
	Cytoplasmic or negative	16	11	11		11	5	
APC					0.588			0.969
		17	9	8		8	9	
		47	29	18		20	27	
Cyclin D1					0.717			0.842
		22	14	8		10	12	
		42	24	18		18	24	
ER					**0.021**			0.227
	Positive	38	27	11		19	19	
	Negative	26	11	15		9	17	
PR					0.847			0.874
	Positive	35	20	15		15	20	
	Negative	29	18	11		13	16	
Total		64	38	26		28	36	

*Chi square test or Fisher's exact test (statistically significant p<0.05).
M, methylated; U, unmethylated; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor.

lent to that of the marker (7 ng/μL) were scored as methylated.

Immunohistochemical staining and interpretation

Paired cores from the neoplastic lesions and the adjacent normal parenchyme of the prepared paraffin blocks were selected and then tissue microarray blocks were constructed. Immunohistochemical staining using diaminobenzidine as the chromogen was performed by an automated staining system (Autostainer Plus, Dako, Denmark). The antibodies that were used were Wnt-1 (G-19, goat polyclonal; Santa Cruz Biotechnology, CA, USA; 1:100 dilution), β-catenin (14/beta-catenin, mouse monoclonal; Transduction Laboratories, KY, USA; 1:200 dilution), APC (C-20, rabbit polyclonal; Santa Cruz Biotechnology; 1:200 dilution), cyclin D1 (DSC-6, mouse monoclonal; Santa Cruz Biotechnology; 1:50 dilution), E-cadherin (HECD-1, mouse monoclon-

al; Zymed, CA, USA; 1:100 dilution), estrogen receptor (ER; ID5, mouse monoclonal; Dako; 1:200 dilution), progesterone receptor (PR; PgR636, mouse monoclonal; Dako; 1:200 dilution) and c-erb B2 (rabbit polyclonal; Zymed; 1:50 dilution). Biotinylated sheep anti-mouse or anti-rabbit IgG (Dako) or donkey anti-goat IgG (Santa Cruz Biotechnology) was used as a secondary antibody.

ER and PR were considered as increased or positive when the proportion of cells that stained positive was above 10%. c-erb B2 was scored on a 0-3+ scale: 0 was, <10% of cells with membrane staining; 1+ was, faint, barely perceptible membrane staining in ≥10% of cells; 2+ was, weak to moderate membrane staining in ≥10% of the cells; 3+ was, strong, complete membrane staining in ≥10% of the cells. A score of 2+ or 3+ was considered as positive. The other immunohistochemical contents were scored depending on the extent and intensity of staining, as was described previously.[20] In brief, the intensity of staining was graded according to a 4-tiered scale of 0 to 3 (with 3 as the most intense staining). The extent of positive immunoreactivity was graded according to the percentage of stained cells in the region of interest: 0 points for 0%, 1 point for <20%, 2 points for 20-50%, and 3 points for >50%. An overall score was obtained from the sum of the intensity and the extent of the positive staining. Cases with a final score of more than three were defined as positive. We also examined abnormal cytoplasmic and/or nuclear localization of β-catenin. Two independent, experienced pathologists graded all the sections without any knowledge of the patients' clinical statuses.

Statistical analysis

The data was analyzed with Statistical Package Service Solution software (SPSS 10.1 for Windows, SPSS Inc., IL, USA). The correlations between the immunoprofile or methylation status and the clinical information were analyzed using the chi square test or Fisher's exact test (2-tailed). Statistical significance was defined as p-value <0.05.

RESULTS

The methylation status of *APC* and *CDH1* and its correlation with the other factors

APC hypermethylation was frequently found in the cases from young age patients (p=0.045) (Table 1). Hypermethylation of the APC and E-cadherin genes was common in breast cancer (59% and 44%, respectively). Of the 38 cases (60%) that showed *APC* promoter CpG island hypermethylation, 29 (76%) were IDCs and nine (24%) were ILCs (Fig. 1A) (Table 1). *CDH1* hypermethylation was observed in 28 (44%) cases, of which 18 cases were IDC and 10 cases were ILC (Fig. 1B) (Table 1). *CDH1* hypermethylation was significantly more frequent in the lobular carcinomas (10/12, 83%) than in the ductal carcinomas (18/52, 35%) (p=0.002), while *APC* hypermethylation did not show any significant difference between the IDCs (29/52, 56%) and the ILCs (9/12, 75%) (p=0.225). The methylation of the APC gene and its protein expression were not associated (p=0.588). *APC* methylation was significantly more frequent in the ER-positive cases (27/38, 71%) than in the ER-negative tumors (11/26, 42%) (p=0.021) (Table 1). *CDH1* methylation showed positive correlation with the tumor stage (p<0.001) (Table 1). *CDH1* hypermethylation was inversely correlated with the membranous expression of E-cadherin (p=0.042) and β-catenin (p=

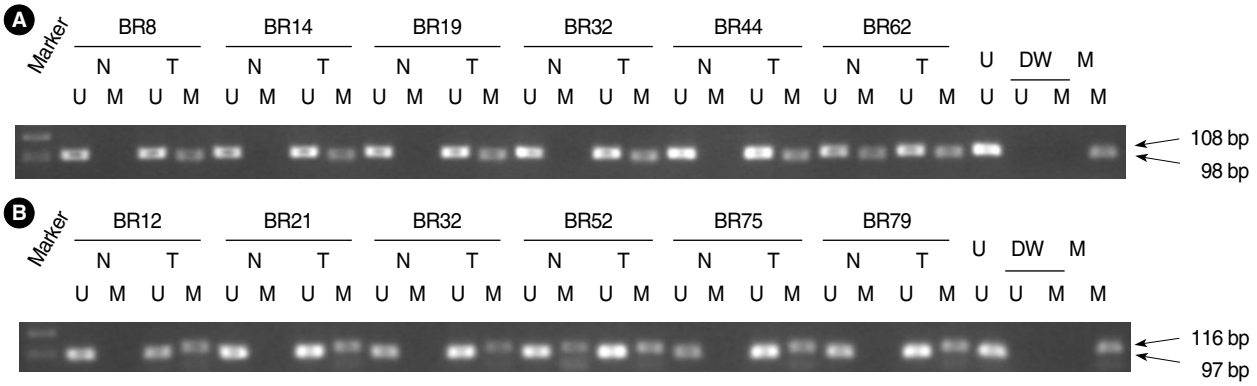

Fig. 1. Methylation specific PCR for CpG islands of *APC* (A) and *CDH1* (B) by methylation specific PCR.
Lane 1, marker; lanes 2-22, normal and tumor cases; U, unmethylated; M, methylated; lane 26, universal unmethylated DNA; lanes 27 and 28, distilled water; lane 29, universal methylated DNA.

0.02) (Table 1). When each subtype was analyzed individually, *CDH1* methylation was inversely correlated with the E-cadherin expression in the IDCs. All the 10 cases of ILC with *CDH1* hypermethylation showed the loss of E-cadherin (data not shown). All the other clinicopathological parameters did not show any correlation with the methylation of the APC gene or the E-cadherin gene nor with their corresponding protein expressions.

Abnormal β-catenin expression and its correlation with the other factors

β-catenin was maintained on the cell membrane in 48 (75%) of the 64 cases (Fig. 2A), but 16 cases (5 IDCs and 11 ILCs) showed a cytoplasmic accumulation or a complete loss of β-catenin (Fig. 2B). No obvious nuclear expression of β-catenin was found in our cases. Of the 12 cases of ILC, five cases showed cytoplasmic staining and six cases lost their β-catenin stainability. This abnormal staining of β-catenin was significantly more frequent in the ILC cases than in the ductal carcinomas ($p<0.001$) (Table 2). An abnormal expression of beta-catenin was associated with none of the other canonical Wnt pathway molecules, such as the APC, Wnt-1 or cyclin D1 expression, nor with the other clinical parameters such as the grade or stage, or the ER and PR statuses (data not shown). E-cadherin was positive in 47 of the 52 IDC cases. In contrast, all 12 ILCs were negative for E-cadherin ($p<0.001$) (Fig. 2C, D) (Table 2). An abnormal β-catenin expression (loss or cytoplasmic) was associated with the loss of E-cadherin ($p<0.001$), but not with the APC expression ($p=0.142$) (Table 3). When the ILCs and IDCs were analyzed individually, the abnormal expression of β-catenin correlated with the loss of E-cadherin, and particularly in the ILCs. In the IDCs, all the five cases with loss of E-cadherin retained the normal membranous β-catenin expression (data not shown).

Expression of the other Wnt pathway molecules

Forty-seven (73%) tumors revealed the loss of an APC expression (Fig. 2E). An APC expression was not associated with an abnormal β-catenin expression ($p=0.142$) (Table 3). Cyclin D1 was positive in 22 cases (34%) (Table 2). Wnt-1 was expressed in 22 tumors (34%), and its positivity rate was not related to the tumor type ($p=0.482$) (Fig. 2F).

DISCUSSION

The Wnt signaling pathway has been a hot topic in the field of oncology, as well as in embryology and stem cell biology. Nevertheless, the results of the research on tumorigenesis in a variety of human organs have been inconsistent. In human breast

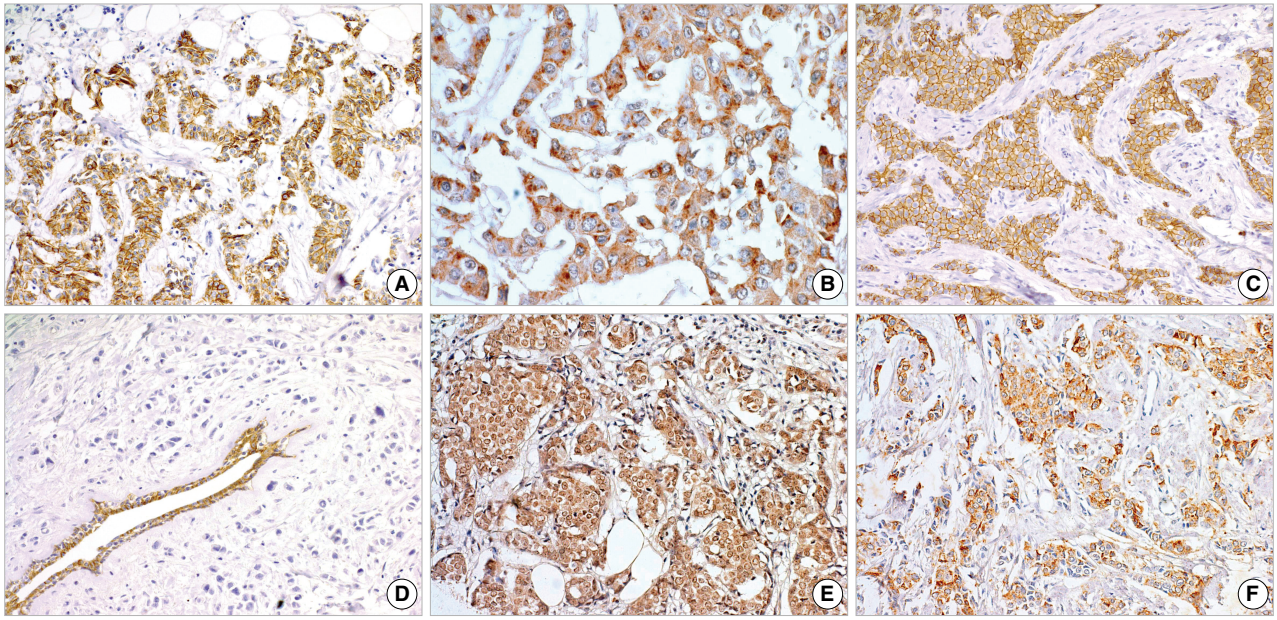

Fig. 2. Immunohistochemical staining patterns of Wnt related markers in mammary carcinomas. β-catenin usually enhances epithelial membrane (A). In contrast to the normal membranous pattern, some cases abnormally express β-catenin in the cytoplasm in invasive ductal carcinoma (B). E-cadherin is preserved on the membrane of ductal carcinoma cells (C), but on the other side E-cadherin is totally lost in lobular carcinoma cells (D). APC (E) and Wnt-1 (F) are expressed in the cytoplasm of invasive ductal carcinoma cells.

Table 2. Comparison of immunohistochemical expression of Wnt/β-catenin pathway-related markers according to the type of invasive mammary carcinomas

Immunohistochemical markers		IDC (n=52)	ILC (n=12)	p-value[a]
E-cadherin				**<0.001**
	Positive	47	0	
	Negative	5	12	
β-catenin				**<0.001**
	Membranous	47	1	
	Cytoplasmic or negative	5	11	
APC				1.431
	Positive	14	3	
	Negative	38	9	
Wnt-1				0.482
	Positive	19	3	
	Negative	33	9	
Cyclin D1				0.482
	Positive	19	3	
	Negative	33	9	
ER				**0.012**
	Positive	27	11	
	Negative	25	1	
PR				**0.027**
	Positive	25	10	
	Negative	27	2	
c-erb B2				**0.003**
	Positive	24	0	
	Negative	28	12	

[a]Chi square test or Fisher's exact test (statistically significant p<0.05).
IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor.

Table 3. Comparison of β-catenin expression with E-cadherin and APC in the invasive mammary carcinomas

Immunohistochemical markers		β-catenin		p-value[a]
		Membranous	Abnormal	
E-cadherin				**<0.001**
	Positive	42	5	
	Negative	6	11	
APC				0.142
	Positive	15	2	
	Negative	33	14	

[a]Fisher's exact test (statistically significant p<0.05).

tissue, Wnt and its related molecules, including E-cadherin and β-catenin, are known to affect the growth and function of the mammary glands, and the destruction of their delicate interaction via some potential mechanisms can result in mammary neoplasia.[21]

ILC is a minor malignant tumor that makes only 5-15% of all invasive breast cancers, and ILC presents with a unique histology and clinical features, including bilaterality and muticentricity.[22,23] In a previously reported larger-scale study on mammary lobular carcinoma, the tumor methylation rate of *CDH1* was 41% (vs 83% in the present study).[11] This result might be caused by the differences in accuracy of microdissection or the additive molecular changes, including genetic mutations or allelic losses. In the present study, E-cadherin loss was found in all 12 ILC cases; 10 of these cases showed epigenetic changes of *CDH1*, while 35% of the IDCs displayed methylation of *CDH1*. This finding shows that *CDH1* promoter hypermethylation is a significantly more common feature in mammary lobular carcinoma than in the ductal carcinomas. The epigenetic alteration of the E-cadherin gene was associated to the loss of the E-cadherin expression, and this result is concordant with the previous studies.[11,12]

Despite the relatively high frequency of epigenetic changes of *APC* and *CDH1*, no abnormal nuclear β-catenin localization was identified in any of our cases. The cytoplasmic accumulation of β-catenin without nuclear localization is consistent with the findings of other reports on mammary carcinomas, which suggests that only a small amount of cytoplasmic β-catenin may move to the nucleus, and the subcellular localization of β-catenin might depend on the tumor type.[11,24,25] In our study, the ILCs more frequently exhibited the cytoplasmic accumulation or loss of β-catenin, as compared to the IDCs (92% vs 10%; p<0.001). Additionally, the cytoplasmic localization or loss of β-catenin was closely associated with the loss of E-cadherin, and particularly in the ILCs.

The activation of a hormonal receptor-dependent Wnt pathway was recently reported in human cancer cell lines.[26] This previous study showed that the overexpression of ER and PR downregulates or activates the Wnt pathway via direct or indirect interactions. Our results revealed that the ER overexpression increased as the methylation of *APC* increased. These results are in agreement with those of a previous study, in which ER-negative breast cancers showed lower frequencies of APC methylation.[27]

Lin *et al.* reported that β-catenin is a prognostic marker, while cyclin D1 is one of the molecular targets of the Wnt/β-catenin signaling pathway in human mammary carcinomas.[28] However, there has been controversy over the clinicopathological significance of the β-catenin expression patterns seen on immunohistochemistry.[29] Our results showed that *CDH1* or *APC* methylation was correlated with β-catenin expression, but not with cyclin D1 overexpression. Future molecular analyses must focus on resolving the limitations of immunohistochemical analysis.

The clinical significance of Wnt-1 in human cancers has not yet been well defined. Only one study has thus far been published

on the Wnt-1 expression in human breast cancer tissue. Wong *et al.* found that Wnt-1 signals were detected in most invasive ductal carcinomas (95%), and its signal declined with tumor progression and the histological grade.[14] In the present study, a Wnt-1 expression was detected in 34% of the cases, and this was irrespective of the tumor type. Because our data did not show any significant association among Wnt-1, APC and β-catenin in contrast to the previous study described above, a potentially expanded role of Wnt-1 in mammary oncogenesis needs to be researched and clarified in the future.

Some investigators have reported that the frequency of *APC* mutation appears to be low in breast cancer, in contrast to colon cancer.[30] In addition, allelic losses, which is another mechanism of gene inactivation, rather than gene mutation itself, could also contribute to the inactivation of *APC* in breast cancer.

In conclusion, this study demonstrated that *CDH1* and *APC* promoter hypermethylations were common features both in the mammary lobular carcinomas and in the ductal carcinomas. *CDH-1* methylation was significantly more common in the ILC than in the IDC, and it was associated with the loss of E-cadherin. Abnormal cytoplasmic accumulation or the loss of β-catenin was correlated with the loss of E-cadherin in mammary lobular carcinomas.

REFERENCES

1. Center CCR. Annual report of the central cancer registry in Korea (2002.1-2002.12): Based on registered data From 139 hospitals. Ministry of Health and Welfare. Seoul: 2003.
2. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005; 434: 843-50.
3. Hajra KM, Fearon ER. Cadherin and catenin alterations in human cancer. Genes Chromosomes Cancer 2002; 34: 255-68.
4. Kashiwaba M, Tamura G, Ishida M. Aberrations of the APC gene in primary breast carcinoma. J Cancer Res Clin Oncol 1994; 120: 727-31.
5. Hirohashi S. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. Am J Pathol 1998; 153: 333-9.
6. Moll R, Mitze M, Frixen UH, Birchmeier W. Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 1993; 143: 1731-42.
7. Oka H, Shiozaki H, Kobayashi K, *et al.* Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res 1993; 53: 1696-701.
8. Berx G, Becker KF, Hofler H, van Roy F. Mutations of the human E-cadherin (CDH1) gene. Hum Mutat 1998; 12: 226-37.
9. Baylin SB, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet 2000; 16: 168-74.
10. Jin Z, Tamura G, Tsuchiya T, *et al.* Adenomatous polyposis coli (APC) gene promoter hypermethylation in primary breast cancers. Br J Cancer 2001; 85: 69-73.
11. Sarrio D, Moreno-Bueno G, Hardisson D, *et al.* Epigenetic and genetic alterations of APC and CDH1 genes in lobular breast cancer: relationships with abnormal E-cadherin and catenin expression and microsatellite instability. Int J Cancer 2003; 106: 208-15.
12. Caldeira JR, Prando EC, Quevedo FC, Neto FA, Rainho CA, Rogatto SR. CDH1 promoter hypermethylation and E-cadherin protein expression in infiltrating breast cancer. BMC Cancer 2006; 6: 48.
13. Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 1982; 31: 99-109.
14. Wong SC, Lo SF, Lee KC, Yam JW, Chan JK, Wendy Hsiao WL. Expression of frizzled-related protein and Wnt-signalling molecules in invasive human breast tumours. J Pathol 2002; 196: 145-53.
15. Brown AM. Wnt signaling in breast cancer: have we come full circle? Breast Cancer Res 2001; 3: 351-5.
16. Lee JY, Dong SM, Kim SY, Yoo NJ, Lee SH, Park WS. A simple, precise and economical microdissection technique for analysis of genomic DNA from archival tissue sections. Virchows Arch 1998; 433: 305-9.
17. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 1996; 93: 9821-6.
18. Esteller M, Sparks A, Toyota M, *et al.* Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. Cancer Res 2000; 60: 4366-71.
19. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet 2000; 25: 315-9.
20. Qiao Q, Ramadani M, Gansauge S, Gansauge F, Leder G, Beger HG. Reduced membranous and ectopic cytoplasmic expression of beta -catenin correlate with cyclin D1 overexpression and poor prognosis in pancreatic cancer. Int J Cancer 2001; 95: 194-7.
21. Meniel V, Clarke AR. Wnt-cadherin connections in normal and neoplastic mammary epithelium. J Mammary Gland Biol Neoplasia 2003; 8: 435-47.
22. DiCostanzo D, Rosen PP, Gareen I, Franklin S, Lesser M. Prognosis in infiltrating lobular carcinoma. An analysis of "classical" and variant tumors. Am J Surg Pathol 1990; 14: 12-23.
23. Lesser ML, Rosen PP, Kinne DW. Multicentricity and bilaterality in invasive breast carcinoma. Surgery 1982; 91: 234-40.
24. Dolled-Filhart M, McCabe A, Giltnane J, Cregger M, Camp RL, Rimm DL. Quantitative in situ analysis of beta-catenin expression in breast

cancer shows decreased expression is associated with poor outcome. Cancer Res 2006; 66: 5487-94.
25. De Leeuw WJ, Berx G, Vos CB, *et al.* Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ. J Pathol 1997; 183: 404-11.
26. Faivre EJ, Lange CA. Progesterone receptors upregulate Wnt-1 to induce epidermal growth factor receptor transactivation and c-Src-dependent sustained activation of Erk1/2 mitogen-activated protein kinase in breast cancer cells. Mol Cell Biol 2007; 27: 466-80.
27. Sunami E, Shinozaki M, Sim MS, *et al.* Estrogen receptor and HER2/neu status affect epigenetic differences of tumor-related genes in primary breast tumors. Breast Cancer Res 2008; 10: R46.
28. Lin SY, Xia W, Wang JC, *et al.* Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc Natl Acad Sci U S A 2000; 97: 4262-6.
29. Wijnhoven BP, Dinjens WN, Pignatelli M. E-cadherin-catenin cell-cell adhesion complex and human cancer. Br J Surg 2000; 87: 992-1005.
30. Furuuchi K, Tada M, Yamada H, *et al.* Somatic mutations of the APC gene in primary breast cancers. Am J Pathol 2000; 156: 1997-2005.