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Chapter IX

Topoisomerase II-Alpha Index Predicts the Efficacy of Anthracycline-Based Chemotherapy for Breast Cancers

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Abstract

DNA topoisomerase II-alpha (TOPOIIα) has been reported that its gene copy number or protein expression may be predictive of Anthracycline-based chemotherapy or patient's prognosis in breast cancers. Our data indicated the breast cancers with TOPOIIα index ≧ 25 % regressed more effectively than those with TOPOIIα index < 10 % on histology by Anthracycline-based chemotherapy. TOPOIIα index of TOPOIIα gene-deleted tumors did not differ from that of TOPOIIα gene-amplificated or normal tumors. TOPOIIα index, not its gene amplification, is a useful marker of Anthracycline-based chemotherapy in breast cancers.

Keywords: DNA Topoisomerase IIα, Index, Anthracycline, Effect prediction, Ki67

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DNA Topoisomerase IIα

DNA topoisomerase is the generic name for an enzyme that severs and reconnects one or both strands of a double-stranded DNA. The enzyme that severs only one of the two strands of a double-stranded DNA is classified as type I, while that which severs both strands is classified as type II. Type II enzyme forms a severing complex by binding to a double-stranded DNA after forming a homodimer. It becomes stable in the presence of Mg^{++} and ATP [1, 2]. Type II enzyme exists in the nucleus and can be classified into α and β, which are expressed from a different gene. Although on the N-terminal side 3/4 of TOPOIIβ is highly homologous with TOPOIIα, it has a different C-terminal domain, and the activity control mechanism exists in this domain.

DNA topoisomerase IIα (TOPOIIα) gene (molecular weight: 170 kDa) is located in the domain 17q21–22, which is near the human epidermal growth factor receptor type 2 (HER2/neu) gene (17q12–21; molecular weight: 185 kDa).

TOPOIIα Index

TOPOIIα protein is a cell proliferation-related antigen, which expresses in the S and G2/M phases during the normal cell cycle. TOPOIIα protein increases by a factor of two to three times in the G2/M phase. Particularly, it expresses most in highly proliferative cells. TOPOIIβ, which has a molecular weight of 180 kDa, constantly exists in any cell and at any phase of the cell cycle; however, its correlation with the therapy-related secondary carcinogenesis has been reported [2, 3]. Although the TOPOIIα index relates to the Caspase-3 index ($p < 0.05$) and the Ki67 index ($p < 0.01$), it is not related to the PCNA index (Figures. 1, 2) [4]. The Ki67 nuclear antigen is not expressed at the silent period (G0); however, it is expressed throughout the cell cycle (i.e., G1, S, G2, and M phases). Ki67 serves as a proliferation marker and a tumor prognostic factor. Although PCNA is also a proliferation-related antigen, its synthesis level begins to increase inside the nucleus at the end of the G1 phase just before the start of DNA synthesis, it maximizes in the S phase and decreases in the G2/M phase. Thus, the expression index of the TOPOIIα protein, which expresses in the S and G2/M phases, only relates to the Ki67 index and not to the PCNA index, even though they are the same cell proliferation-related antigens. Although it is not totally clear why the protein index of Caspase-3, which is a protease that works in the final stage of apoptosis, loosely relates to that of TOPOIIα, one must pay careful attention when evaluating apoptosis for the evaluation of the therapeutic effects of Anthracycline-based chemotherapy, which induces apoptosis from TOPO II inhibition [5–8]. Moreover, the TOPOIIα index not only indicates the proliferation activity but may also relate to the quantitative changes of cancer cells [8, 9].

Ki67 Index

TOPOIIα and Ki67 are often compared as prediction makers for the therapeutic effects of Anthracycline-based chemotherapy. However, because the methods of positive/negative

classification used in the reports vary to a small extent, it is necessary to be careful when comparing the positive rates or evaluating the significance of the measurements. According to Nakopoulou et al., 25% of the invasive breast cancer subjects experienced the TOPOIIα protein expression in more than 10% of the tumor cells.

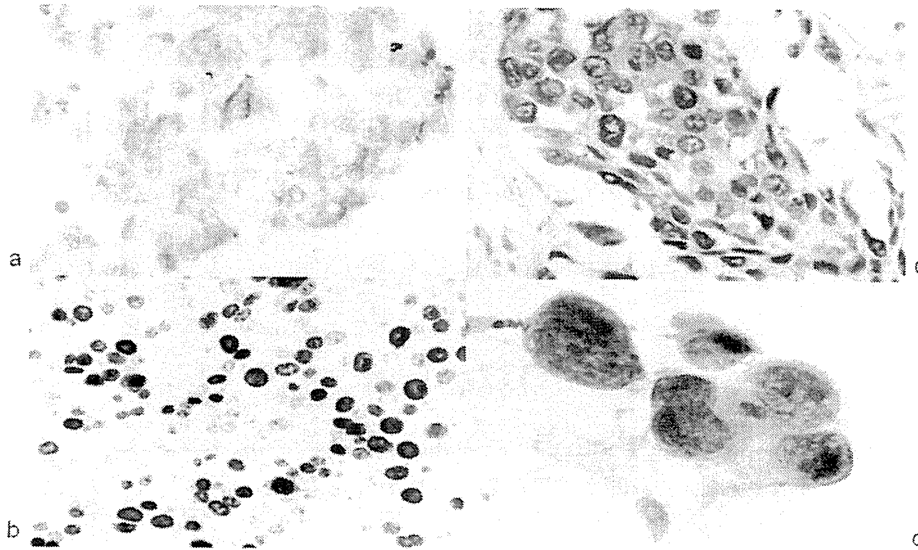


Figure 1. Immunochistochemical findings and cytological atypia of breast cancer cells. (a: Caspase 3 index- 44.8%, b: Ki67 index-18.8%, c: PCNA index-88.7%, d: nuclar atypia grade 3 by Robinson's classification).

The expression of TOPOIIα protein had correlations with nuclear atypia, the Ki67 index, and the p53 index of the tumor cells, as well as hormone receptor non-expression and HER2 protein overexpression in the tumor cells [10]. We investigated the correlation of the positive rate using the 203 infiltrating duct carcinomas as subjects, and reported that the TOPOIIα index had correlations with nuclear atypia (Robinson grade) [11] as well as with the Ki67 index, Caspase-3 index, hormone receptor non-expression, and HER2 gene amplification (Table 1) [4]. However, according to the study conducted by Petit et al., the following facts have been discovered: Of all the factors (nuclear atypia, hormone receptor, Ki67 index, HER2 protein expression, TOPOIIα protein expression, HER2 gene amplification, and TOPOIIα gene amplification), hormone receptor non-expression and the Ki67 index of 20% or greater are effective for the clinical CR (complete response) prediction for neoadjuvant chemotherapy or primary systemic therapy for breast cancer using an anthracycline; and while nuclear atypia has a correlation with the pathological CR prediction, neither protein expressions nor gene amplifications of HER2 and TOPOIIα had any correlation with the therapeutic effect [12]. Meanwhile, Tinari et al. have reported that in the breast cancer cases treated with neoadjuvant chemotherapy or primary systemic therapy using an anthracycline, while HER2 protein and TOPOIIα protein expressions had correlations with the therapeutic effect, neither nuclear atypia nor the Ki67 index had any correlation with the therapeutic effect [13].

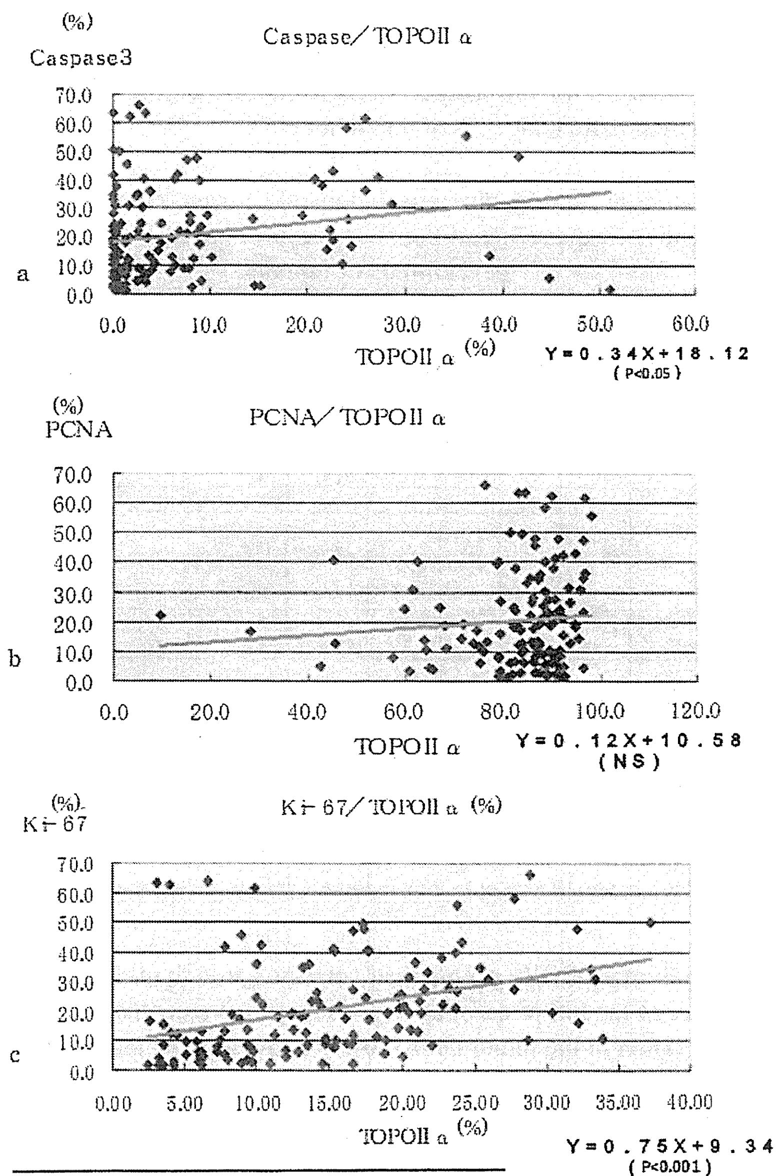


Figure 2. Relations of TOPOIIα index to Caspase 3 index (A), PCNA index (B), and Ki67 index (C), with statistical significance in A and C.

TOPOIIα and HER2 Genes

HER2 genes are the cancer genes that amplify most frequently in breast cancer. One must also be careful when interpreting the results of gene amplification evaluation on these genes because the standards used for evaluation in the previous reports vary. The HER2 gene amplification measured through the fluorescence in situ hybridization (FISH) method can be indicated in the form of signal comparison with CEP17 (Chromosome 17 centromere) (Figure 3). It has been reported that the frequency of occurrence of the signal rate of ≥2.0 is 18%–

29.4% [14–17]. Although monoclonal antibody trastuzumab (Herceptin) is effective in only 23%–26% of the breast cancers having overexpressed HER2 proteins during the simple substance treatment against HER2 proteins, when combined with other anticancer agents the effect will increase up to 50% [17].

Table 1. Relationships among clinicopathological characteristics, topoisomerase II alpha, HER2, Ki67, Caspase 3, and hormon receptors in 203 invasive ductal breast carcinomas of females

Clinico-	Number		Tumor	Imunochistochemistry						FISH	
pathological	of	Age	size	ER score	PgR score	Hercep score	Ki67(%)	TOPOII(%)	Caspase3 (%)	HER2/CEP	TOPOIIα/ CEP
characteristics	cases		cm	0–3	0–3	0–3	%	%	%	index	index
All	203	60,2	2,3	1,7	1,5	1,0	19,0	18,5	6,3	2,9	1,5
Tumor size											
pT1	112	60,7	1.4a	1,8	1,5	0,9	18,4	18,1	5,6	2,8	1,4
pT2	79	58,9	2,9	1,7	1,5	1,0	20,0	19,2	7,1	2,9	1,6
pT3,4	12	63,3	6,7	1,8	1,3	1,4	17,9	17,7	7,6	4,3	1,7
Pathological stage											
I	76	61,0	1.3a	1,8	1,5	0,8	17,5	16,8	5,9	2,5	1,5
IIA	68	60,3	2,3	1,8	1,4	1,2	18,9	20,0	5,8	3,4	1,6
IIB	44	58,9	3,0	1,7	1,7	0,9	20,3	19,3	7,4	2,7	1,4
III, IV	12	60,3	6,1	1,7	1,1	1,5	18,1	19,4	8,7	4,3	1,7
LN meta (pT1&2)											
absent	110	60,7	1.8a	1,8	1,5	0,9	17,9	17,4	6,2	2,8	1,5
present	78	59,2	2,3	1,7	1,5	1,1	19,8	20,3	6,3	3,0	1,5
Robinson grade											
1	92	62.5b	2,1	2.1a	1.8a	0.8a	14.5b	14.9a	3.4c	1.9b	1,4
2	82	59,0	2,4	1,6	1,4	1,1	20,8	20,5	7,7	3,3	1,6
3	24	54,2	2,5	0,7	0,6	1,6	30	30,6	12,6	6,1	1,7

Positive cells at ER, PgR score: none, 0; <10%, 1; 10–50%, 2; ≥50%, 3.
a, $P<0.001$; b, $P<0.01$; c, $P<0.05$.

Jarvinen et al. conducted a study deeming the TOPOIIα / CEP17 signal rate of ≥1.5 as amplification and that of ≤0.7 as deletion, and reported that the TOPOIIα genes were either amplified or deleted in 90% of the breast cancers, in which HER2 genes had been amplified [8, 9]. According to the report by Coon et al. that deemed the signal rate of ≥2.5 as amplification, 23% of the invasive breast cancers showed HER2 gene amplification. Of these

23% tumors, TOPOIIα gene amplification was evident in 67.7%. However, no deletion was observed in either the HER2 genes or TOPOIIα genes [18]. Knoop et al. reported that when deeming the signal rate of ≥2.0 as amplification and that of <0.8 as deletion, the HER2 genes amplified in 29.4% of the breast cancers. In 32.5% of such tumors, the TOPOIIα genes also amplified. Moreover, deletion was observed in 23.6% of the HER2 gene-amplified breast cancers.

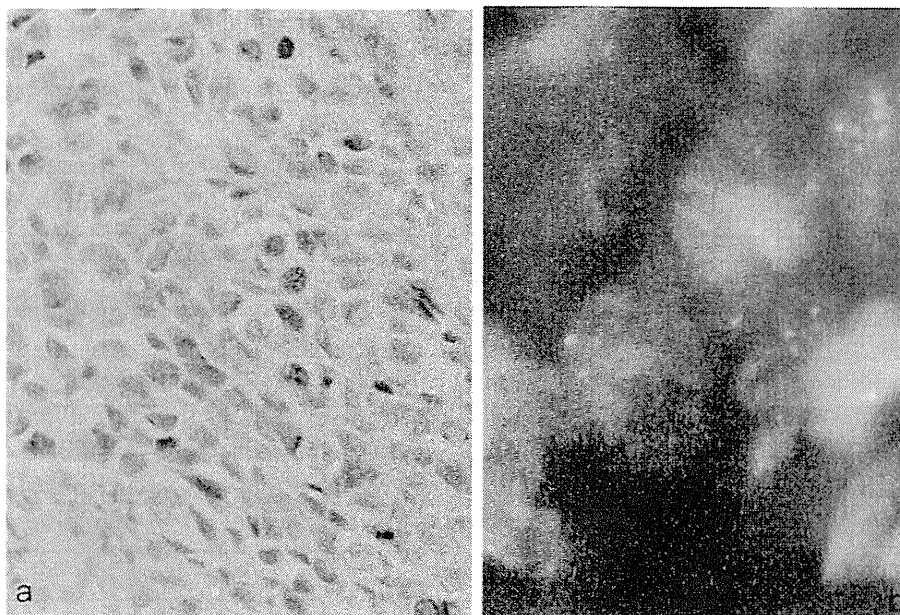


Figure 3. TOPOIIα index of 16.3% (a) and aneuploidy of TOPOIIα gene (b: TOPOIIα / CEP17 = 1.14). Index was evaluated under 40× objective with respect to the positive cell ratio calculated based on the selected five visual fields that are most frequently stained.

In our study, when deeming the signal rate of ≥2.0 as amplification and that of <0.8 as deletion, HER2 gene amplification was observed in 26.6% of the infiltrating duct carcinomas. Of these tumors, TOPOIIα gene amplification and deletion were observed in 25.9% and 11.1% of the tumors, respectively. In addition, when deeming the signal rate of ≥1.5 as amplification, the HER2 gene amplification was observed in 39.9% of the tumors. Of these tumors, TOPOIIα gene amplification and deletion were observed in 51.9% and 8.6% of the tumors, respectively. Moreover, when the HER2 genes were normal (signal rate ranging from 0.8 to 1.5), the TOPOIIα gene amplification was observed in 1.7% at the signal rate of ≥2.0 and in 10.1% at the signal rate of ≥1.5, while deletion was observed in 0.8% [4].

At present, in Japan, the classification of gene amplification is being unified into the HER2 Gene Amplification Standards published by the American Society of Clinical Oncology (ASCO) in 2007. According to these standards, the signal rate of ≥2.2 is deemed as amplification, ranging from 0.8 to 1.8 is deemed as normal, and that of <0.8 is deemed as deletion. The signal rate ranging from 1.8 to 2.2 is classified under the new concept of "equivocal" (borderline region) [15]. Table 1 shows the classification of the results of our study previously described based on these standards. Compared to the data having the signal rate of ≥2.0, the amplification positive rate decreased slightly.

Topoisomerase Inhibition

There are three types of topoisomerase inhibitors: type I inhibitor, type II inhibitor, and dual inhibitor (which inhibits both types). There are two classifications of type II inhibitors: One inhibits the reconnection of the severed double-stranded DNA caused by TOPO II, in doing so it stabilizes the severing complex; and the other inhibits DNA synthesis through the intercalation (bonding) of the double-stranded DNA. Those that display both actions are called intercalators, and those that only show the TOPO II inhibitor action are called non-intercalators [1]. TOPOII inhibitors include anthracyclines (doxorubicin, epirubicin, mitoxantrone, amsacrine, and actinomycin D) as intercalators and epipodophyllotoxins (etoposide and teniposide) as non-intercalators [19].

Anthracyclines, which are intercalators, serve as substrates of P-glycoprotein, and therefore, transfer into cells through passive diffusion [1], where they cause DNA damage due to the TOPO II inhibition and thereby induce apoptosis. They are secreted by P-glycoprotein existing in the brain-vascular barrier. P-glycoprotein high expression, TOPO II protein low expression, and gene mutation are all related to the resistance against the Anthracycline-based chemotherapy.

In addition, because the cardiotoxicity of anthracyclines is irreversible [20], if anthracyclines are used in combination with trastuzumab, cardiac disturbance occurs with high frequency [21]. When administering an anthracyclines, the left ventricular ejection function should be measured using echography and scintigraphy on a regular basis. If the ejection fraction deteriorates, it is important to immediately stop administering the anthracyclines [22].

TopoIIα Genes, Protein Expression, and TopoIIα Inhibitor Sensitivity

TOPOIIα is a molecular target of TOPO II inhibitors, which inhibit the function of TOPOIIα proteins. Thus, the sensitivity of TOPOIIα inhibitors depends on the level of TOPOIIα protein expression of the cancer cells. In other words, a tumor having a low TOPOIIα protein concentration has lower TOPOIIα inhibitor sensitivity than a tumor having a higher TOPOIIα protein concentration. Moreover, in the cancer cells having a high concentration TOPOIIα protein level, hormone receptor non-expression, HER2 protein overexpression, p53 genetic abnormality, DNA aneuploidy, and poor differentiation are observed [9]. Meanwhile, with regard to the TOPOIIα genes, Knoop et al. reported that both TOPOIIα gene amplification and deletion were effective markers for the prediction of anticancer agents, including epirubicin [14]. Epirubicin is one of anthracyclines in which the cardiotoxicity has been reduced [1]. Knoop et al. investigated the HER2 genes and TOPOIIα genes of breast cancers that had been surgically removed prior to the implementation of the CMF (cyclophosphamide, methotrexate, fluorouracil) and CEF (cyclophosphamide, epirubicin, fluorouracil) treatments, using the FISH method. The researchers then investigated the HER2 protein expression immunohistochemically. As a result, it was found that although the state of the HER2 genes had no correlation with the therapeutic effect, the abnormality of

the TOPOIIα genes (i.e., amplification and deletion) correlated with the patient's disease-free survival and the overall increase in the survival rate.

With regard to the TOPOIIα gene deletion, Jarvinen et al. have reported that under the condition of HER2 gene amplification, it occurs with frequency similar to that of amplification (amplification ≥1.5, deletion ≤0.7), and that the TOPO II inhibitor sensitivity decreases [8]. In our study, when deeming the signal rate of ≥1.5 as amplification, HER2 gene amplification was observed in 39.9% of all the infiltrating duct carcinomas. While TOPOIIα gene amplification occurred in 51.9% of the 39.9% infiltrating duct carcinomas, TOPOIIα gene deletion occurred in only 8.6% [4]. Park et al. also investigated breast cancers, deeming the signal rate of ≥1.5 as amplification and that of ≤0.75 as deletion. As a result, HER2 gene amplification was observed in 8.5% of the tumors. Of the 8.5% tumors, TOPOIIα gene amplification occurred in 18 tumors (75%) and deletion occurred in 25% of the tumors. Based on these findings, they reported that TOPOIIα gene deletion does not occur as frequently as amplification [23]. With regard to HER2 gene amplification, the signal rate of ≥2.0 is usually deemed as amplification in the conventional evaluation method. However, because it has been proposed that having the signal rate of ≥2.2 or more than six copies should be deemed as amplification from 2007 [15], it is necessary to organize the frequency of TOPOIIα genetic abnormalities according to the new classification.

Table 2. HER2 gene and TOPOIIα gene in 203 breast cancers (ASCO 2007)

HER2/CEP			TOPOIIα/CEP		
	Amplification ≥2.2	Equivocal 1.8-2.2	Normal 0.8 - 1.8	Deletion <0.8	All
Amplification ≥2.2	12 (23.5%)	4 (7.8%)	29 (56.9%)	6 (11.8%)	51(25.1%)
Equivocal 1.8-2.2	1 (6.7%)	3 (20.0%)	10 (66.7%)	1 (6.7%)	15 (7.4%)
Normal 0.8 - 1.8	3 (2.2%)	6 (4.5%)	124 (92.5%)	1 (0.7%)	134 (66.6%)
Deletion <0.8	0	0	1 (33.3%)	2 (66.7%)	3 (1.5%)
All	16 (7.9%)	13 (6.4%)	164 (80.8%)	10 (4.9%)	203

Table 3. Relationships between HER2 gene and TOPOIIα gene status

HER2/CEP			TOPOIIα/CEP		
	Amplification ≥2.0	Gain 1.5-2.0	Normal 0.8 - 1.5	Deletion <0.8	All
Amplification ≥2.0	14 (25.9%)	13 (24.1%)	21 (38.9%)	6 (11.1%)	54 (26.6%)
Gain 1.5-2.0	5 (18.5%)	10 (37.0%)	11 (40.7%)	1 (7.4%)	27 (13.3%)
Normal 0.8 - 1.5	2 (1.7%)	10 (8.4%)	106 (89.1%)	1 (0.8%)	119 (58.6%)
Deletion <0.8	0	0	1 (33.3%)	2 (66.7%)	3 (1.5%)
All	21 (10.3%)	33 (16.3%)	139 (68.5%)	10 (4.9%)	203

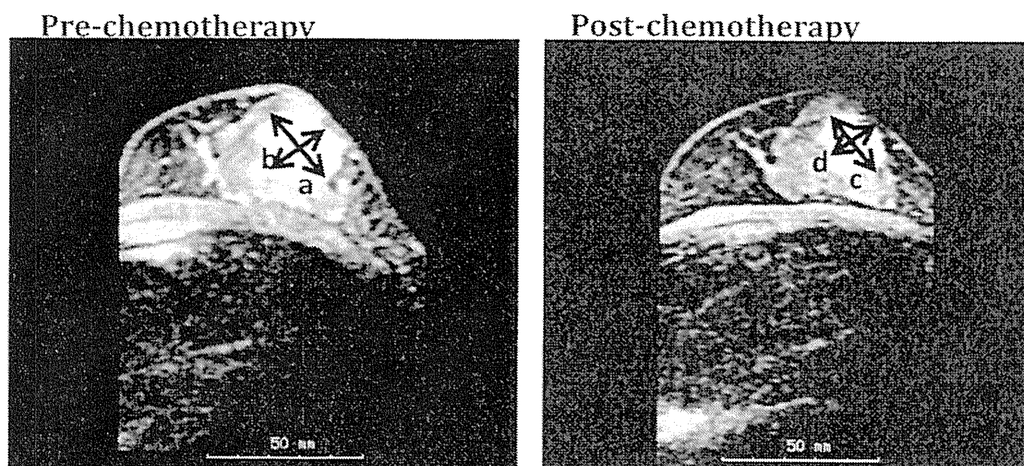
In our study, when deeming the signal rate of ≥2.0 as amplification, TOPOIIα genetic abnormalities (i.e., amplification and deletion) occurred in 37.0% of the HER2-amplified breast cancers (Table 3). Moreover, when deeming the signal rate of ≥2.2 as amplification, the same phenomena occurred in 35.3% of the same cancers (Table 2). In addition, although it had been shown in previous reports that TOPOIIα genetic abnormalities could be observed only in HER2-amplified breast cancer (24), it was subsequently reported that such genetic abnormalities can also be observed in HER2 non-amplified tumors. Knoop et al. has observed TOPOIIα genetic abnormalities in 56.9% of the HER2-amplified tumors (with the signal rate of ≥2.0) and in 7.6% of the HER2 non-amplified tumors (14). Park et al. also found TOPOIIα genetic abnormalities in 0.95% of the entire group of HER2 non-amplified tumors [23]. In our study, when deeming the HER2 genetic normality as 0.8–1.8, amplification was observed in 2.2% of the tumors and deletion was observed in 0.7% of the tumors (Table 2).

The TOPO II inhibitor sensitivity depends on the level of TOPOIIα protein expression of the cancer cells. Table 4 indicates the results of comparison among the TOPOIIα gene, the TOPOIIα index, the Ki67 index, and the HER2 gene in 172 infiltrating duct carcinomas. There was no difference between the TOPOIIα index of the TOPOIIα gene-deleted tumors and that of other groups. Moreover, there was no difference between the Ki67 index of the TOPOIIα gene-deleted tumor and that of the amplified tumors. Furthermore, the Ki67 index of the TOPOIIα gene amplification cases (signal rate of ≥2.2) significantly increased in comparison to the normal cases (signal rate ranging 0.8–1.8) . We performed the neoadjuvant chemotherapy with anthracyclines for 12 infiltrating ductal carcinomas of the breast and evaluated the tumor reduction rates (Figure 4) after the chemotherapy. As a result, we found a statistically significant ($p = 0.01$) correlation of the tumor reduction rate with the TOPOIIα index, but not with TOPOIIα or HER2 gene amplification (Figure 5). Moreover, among the 28 tumors in which the neoadjuvant chemotherapy with anthracyclines was performed, the correlation between the TOPOIIα index and the tumor regression rates had been evaluated. Consequently, it was observed that the tumor regression rate increased more significantly in the breast cancers in which the TOPOIIα index was evaluated ≥25% than the tumors in which the TOPOIIα index was evaluated <10% (Figure 6). Although these 28 cases included no case of TOPOIIα gene deletion, PR (partial response) with the regression rate of 22% was observed in a single TOPOIIα gene-deletion case (TOPOIIα gene signal rate: 0.69, TOPOIIα protein index: 17.0%, and Ki67 index: 53.5%), on which the clinical image evaluation was conducted after administering the anthracyclines and taxane. These facts suggest the possibility that the TOPOIIα index, immunohistochemically calculated on the tissue obtained prior to the Anthracycline-based chemotherapy, may serve as a prediction factor for the effects of TOPO II inhibitors. In addition, Tinari et al. conducted a study on the breast cancer cases in which neoadjuvant chemotherapy or primary systemic therapy was performed using the anthracyclines. They reported that the HER2 protein expression and TOPOIIα protein expression correlated to the therapeutic effects, and the cases in which the level of TOPOIIα protein expression had increased after the therapy showed significantly low survival rates [13]. Considering this data, when using the TOPO II inhibitor for chemotherapy, it is important to calculate the immunohistochemical TOPOIIα index in the tissues obtained before and after chemotherapy, particularly in terms of prediction for the therapeutic effect of the anthracyclines as well as the prediction of patient prognosis.

Table 4. Comparison of TOPOIIα gene, TOPOIIα Index, Ki67 index and HER2 gene in 172 infiltrating ductal carcinomas of the breast

TOPOIIα	Number	TOPOIIα	Ki67	Number of HER2			
				A	E	N	D
Amplification ≥2.2	15 (8.7%)	19.7%	26.6% [a, b]	11	1	3	0
Equivocal 1.8-2.2	9 (5.2%)	20.8%	15.9% [a]	2	2	5	0
Normal 0.8-1.8	140 (81.4%)	16.9%	18.1% [b]	19	9	111	1
Deletion <0.8	6 (3.5%)	22.9%	29.7%	4	1	1	0

A,Amplification; E,Equivocal; N, Normal; D, Deletion
^{a}P = 0.038(Welch), P = 0^{b}.022(student t)



Reduction rate = (a + b) - (c + d) / a + b

Figure 4. Tumor reduction rate calculating the tumor diameter before and after the chemotherapy.

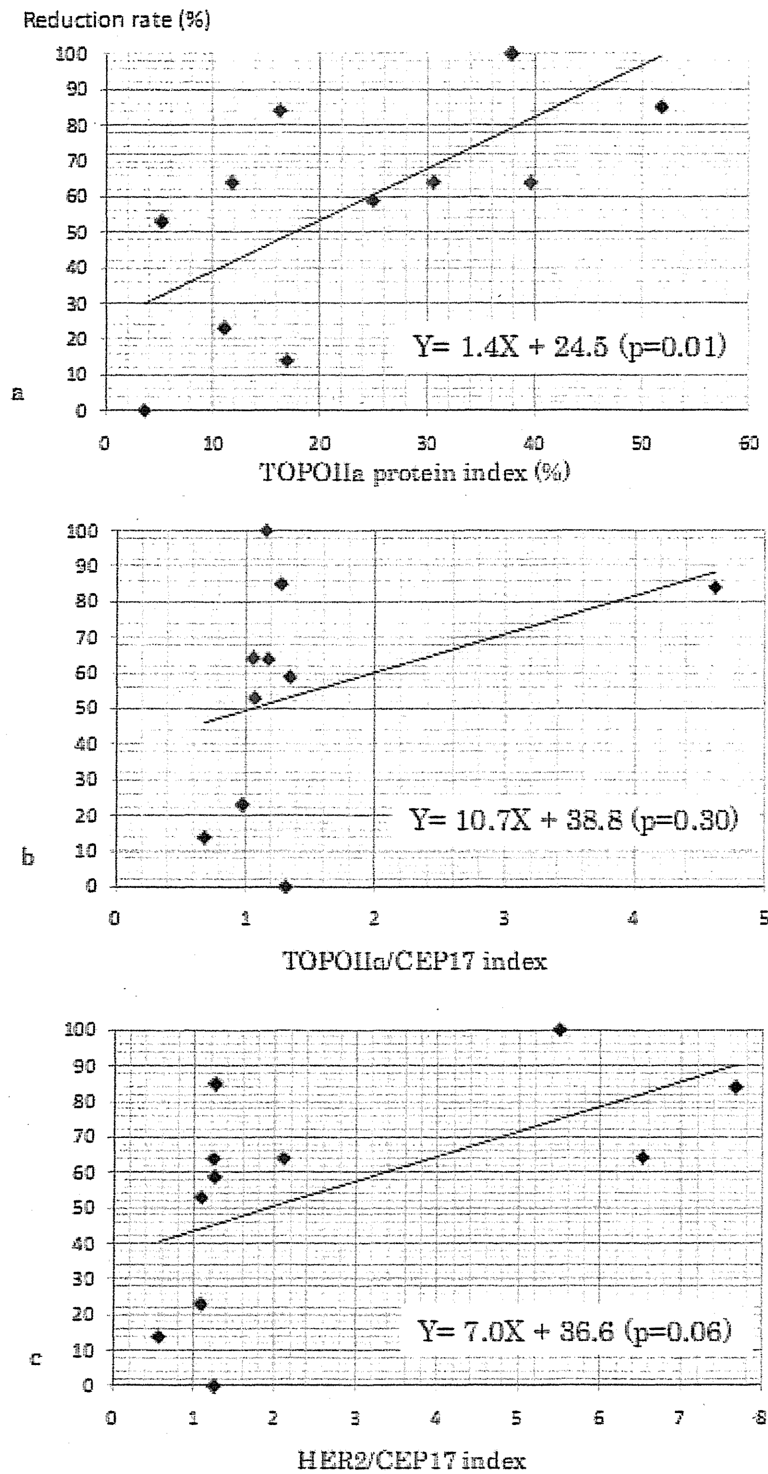


Figure 5. Relations of the tumor reduction rates to the TOPOIIα index (a; $p = 0.01$), TOPOIIα gene (b; $p = 0.30$) and HER2 gene (c; $p = 0.06$).

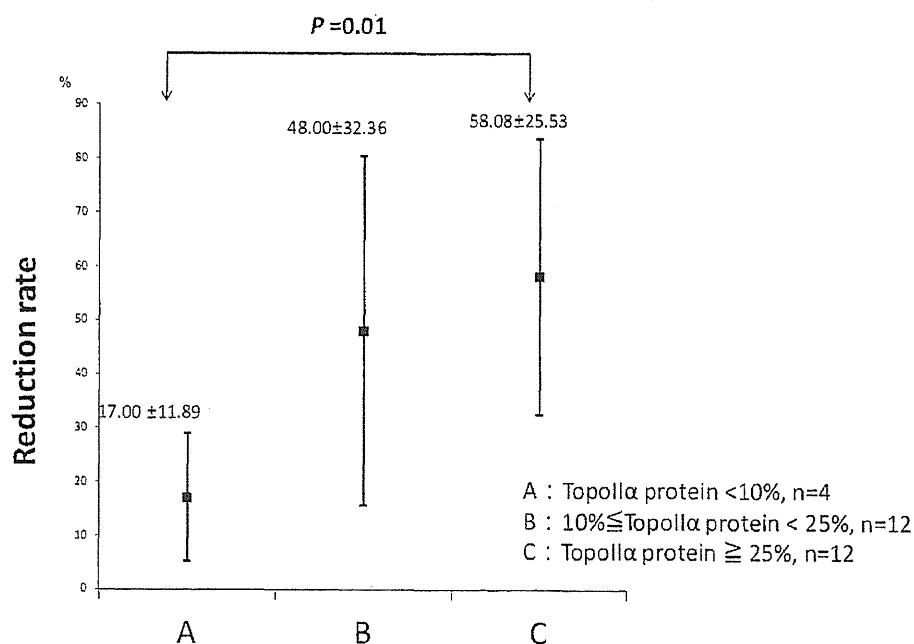


Figure 6. Correlation between the TOPOIIα index and the tumor regression rate.

References

[1] Kato K, et al: Topoisomerase inhibitors. Japanese Clinical Oncology Committee ed.,In; *New Clinical Oncoogy forChemotherapy by Clinical Oncologist*. p274-280, Nankodo, Tokyo, 2008 (in Japanese)

[2] Pommier Y, et al: Topoisomerase II Inhibitors: The Epipodophyllotoxins, Acridines, and Ellipticines. Cancer Chemotherapy and Biotherapy Principles and Practice. (Chabner BA, Longo DL, editors.) p451-475, Lippincott Williams and Wilkins. Philadelphia: 2006.

[3] Toyoda E, et al: NK314, a topoisomerase II inhibitor that specifically targets the alpha isoform. *J. Biol. Chem*. 283:23711-23720, 2008 (in Japanese).

[4] Taniyama K., et al: DNA topoisomerase II alpha. Toi M. ed., In: Basic and Clinic of Breast Cancers for Everyone. p377-388, *IYAKU Journal*, Osaka, 2009 (in Japanese).

[5] Okamoto N., et al. Relations among cellular atypia, proliferative activity and TOPOIIα expression in ductal carcinoma of the breast. *Proceeding of the 59th annual meeting of national hospitals in Japan*. 2005:416 (in Japanese).

[6] Gruber BM, et al: Relationship between topoisomerase II-DNA cleavable complexes, apoptosis and cytotoxic activity of anthracyclines in human cervix carcinoma cells. *Anticancer Res*. 25:2193-2198, 2005.

[7] Chang J, et al: Apoptosis and proliferation as predictors of chemotherapy response in patients with breast carcinoma. *Cancer*. 89:2145-2152, 2000.

[8] Jarvinen TA, et al: Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. *Am. J. Pathol.* 156:839-847, 2000.

[9] Jarvinen TA, et al: HER2/neu and topoisomerase IIalpha--simultaneous drug targets in cancer. *Comb. Chem. High Throughput Screen.* 6:455-470, 2003.

[10] Nakopoulou L, et al: DNA topoisomerase II-alpha immunoreactivity as a marker of tumor aggressiveness in invasive breast cancer. *Pathobiology*. 68:137-143, 2000.

[11] Robinson, I. A.et al: Typing and grading breast carcinoma on fine-needle aspiration: is this clinically useful information? *Diagn Cytopathol.* 13: 260-265, 1995.

[12] Petit T, et al: Comparative value of tumor grade, hormonal receptors, Ki-67, HER2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. *Eur. J. Cancer.* 40:205-211, 2004.

[13] Tinari N, et al: Changes of topoisomerase IIalpha expression in breast tumors after neoadjuvant chemotherapy predicts relapse-free survival. *Clin. Cancer Res.* 12:1501-1506, 2006.

[14] Knoop AS, et al. Retrospective analysis of topoisomerase IIa amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil: Danish Breast Cancer Cooperative Group. *J. Clin. Oncol.* 23:7483-7490, 2005.

[15] Wolff AC, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *Arch. Pathol. Lab. Med.* 131:18-43, 2007.

[16] Pauletti G, et al: Assessment of methods for tissue-based detection of the HER2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J. Clin. Oncol.* 18:3651-3664, 2000.

[17] Taniyama K, et al: Tyrosine1248-phosphorylated HER2 expression and HER2 gene amplification in female invasive ductal carcinomas. Breast Cancer. 15:231-240, 2008.

[18] Coon JS, et al: Amplification and overexpression of topoisomerase II_alpha predict response to anthracycline-based therapy in locally advanced breast cancer. *Clin. Cancer Res.* 8:1061-1067, 2002.

[19] Inoue K. Mechanisim of the anticancer drugs. Ito Y. and Toi M., ed., In: Breast disease-State of arts. p335-337, *ISHIYAKU shuppan*, Tokyo, 2004 (in Japanese).

[20] Katsumata N.: trastuzumab. Japanese Clinical Oncology Committee ed.,In; New Clinical Oncology forChemotherapy by Clinical Oncologist. p312-315, Nankodo, Tokyo, 2008 (in Japanese)

[21] Seidman A, et al: Cardiac dysfunction in the trastuzumab clinical trials experience. *J. Clin. Oncol.* 20:1215-1221, 2002.

[22] Okamoto M., et al: Maintenance therapy 1) How to treat the adverse effect of drugs used for chemotherapy. Japanese Clinical Oncology Committee ed.,In; *New Clinical Oncology for Chemotherapy by Clinical Oncologist.* p716-723, Nankodo, Tokyo, 2008 (in Japanese)

[23] Park K, et al: Topoisomerase II-alpha gene deletion is not frequent as its amplification in breast cancer. *Breast Cancer Res. Treat.* 98:337-342, 2006.

[24] Jarvinen TA, et al: HER2/neu and topoisomerase II_alpha in breast cancer. *Breast Cancer Res. Treat.* 78:299-311, 2003.

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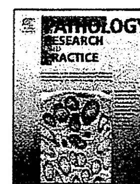

Review Series of the Upper Gastrointestinal Tract

Molecular pathology of gastric cancer: Research and practice

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ABSTRACT

Recent advances in the understanding of molecular stomach carcinogenesis are reviewed. As to molecular events in individual mucin phenotypes of gastric cancer, the CDX2-Reg IV-SOX9 pathway is associated with the intestinal mucin phenotype, while OLFM4 and CLDN18 are novel markers for the gastric phenotype. microRNAs play an important role in epigenetic deregulation in gastric cancer. Many microRNAs are up-regulated and down-regulated, and some of these are associated with histological differentiation and cancer progression. Reduced miR-200 may participate in the genesis of diffuse type gastric cancer by reducing E-cadherin expression. Genetic polymorphism is a crucial endogenous cause and a fundamental factor of cancer risk. PSCA polymorphism alters the susceptibility to diffuse type gastric cancer through modulation of cell proliferation activity. Cancer stem cells possess the capacity for self-renewal and cause the heterogeneous lineages of cancer cells. Cancer stem cells also show resistance to antitumor chemotherapy. Only a minor population of gastric cancer cells reveals the properties of cancer stem cells, and CD44 is one of the markers for gastric cancer stem cells. The origin of gastric cancer stem cells remains to be elucidated.

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Introduction

Gastric cancer is one of the most common cancers worldwide, and mortality due to gastric cancer is second next to lung cancer. Cancer develops as a result of an accumulation of various endogenous and exogenous causes. Dietary factors and *Helicobacter pylori* (*H. pylori*) infection are important exogenous causes for gastric cancer, while many genetic polymorphisms are found to be associated with predisposition to cancer development. Multiple genetic and epigenetic alterations occur in the course of carcinogenesis and progression of gastric cancer [56,58,59,61,62]. These include telomerase activation, genetic instability, and abnormalities in oncogenes, tumor suppressor genes, growth factors, matrix degradation enzymes, cell cycle regulators, cell adhesion molecules, etc. Gastric cancers are histologically classified into "differentiated" and "undifferentiated" types, or "intestinal" and "diffuse" types based on the glandular structure, while these can also be classified into "intestinal" and "gastric" types on the basis of the mucin expression profile [48]. Some of the genetic and epigenetic changes differ depending on the histological type or mucin phenotype. Advances in genomic science have made it possible to uncover detailed mechanisms of molecular stomach carcinogenesis. In recent years, microRNA (miRNA) was believed to play a crucial role in the cellular process by regulating gene expression and to participate deeply in epigenetic alterations in gastric cancer [8]. Furthermore, a recent focus of attention was the presence of "cancer stem cells" among heterogeneous cancer cells and its therapeutic implication [21].

This review describes the molecular carcinogenesis of "intestinal" and "gastric" types of gastric cancer, the importance of miRNAs, the significance of genetic polymorphism, and the role of cancer stem cells in gastric cancer.

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Molecular carcinogenesis of intestinal and gastric type gastric cancer

In addition to the histological classification, such as "differentiated" and "undifferentiated" types, or "intestinal" and "diffuse" types based on glandular structure, gastric cancers are also classified into four types: G (gastric) type, I (intestinal) type, GI (gastric and intestinal mixed) type, and N (neither gastric nor intestinal) type, on the basis of the mucin expression, including MUC5AC, MUC6, MUC2 and CD10 [48]. It has been shown that *sry*-related high-mobility group (HMG) box 2 (SOX2) is stomach-specific, and caudal-type homeobox (CDX) 1 and CDX2 are intestine-specific transcription factors [2,50]. This classification is important, because some molecular events cause differences between the G type and I type; DNA methylation of the mismatch repair gene and microsatellite instability are frequent in G type, and p53 mutation occurs frequently in I type [24,41]. It has also been suggested that in comparison with I type, G type is biologically aggressive [45].

We have performed transcriptome dissection of gastric cancer through serial analysis of gene expression (SAGE) and found several novel genes associated with G type and I type gastric cancers [34,57]. SAGE allows for the global analysis of gene expression in a quantitative manner, without prior knowledge of the exact sequence of the genes. Our SAGE library is one of the largest gastric cancer libraries in the world, and the sequence data are publicly available at SAGEmap (GEO accession number GSE 545, SAGE Hiroshima gastric cancer tissue) [34]. By comparing SAGE data between gastric cancers and normal gastric tissue in combination with quantitative RT-PCR and immunohistochemistry, several genes, such as Reg IV, OLFM4, and CLDN18, were found to show mucin phenotype-specific expression [61].

Reg IV (regenerating islet-derived family, member 4) belongs to the calcium-dependent lectin superfamily, and was originally isolated as a gene up-regulated in inflammatory bowel diseases [18]. Reg IV is expressed in about 30% of gastric cancers, and Reg IV protein is detectable in sera of about 30% of gastric cancer patients [31]. Forced expression of Reg IV in gastric cancer cell lines induced the expression of the phosphorylated form of the EGF receptor, Bcl-2, Bcl-XL, survivin, and the phosphorylated form of AKT. Mucin-like staining of Reg IV in gastric cancer was significantly associated with MUC2-positive I type. CDX2 induced the expression of Reg IV. Therefore, CDX2 is an up-stream regulator for Reg IV expression [61]. SOX9 was found to be one of the down-stream targets of Reg IV. SOX9 is a member of the SOX [*sry*-related high-mobility group box] family of HMG DNA-binding domain transcription factors, and is required for the development and differentiation of multiple cell lineages [40]. Many cancer cells of I type are positive for SOX9, and SOX9-positive tumor cells co-localize with Reg IV-positive cells. CDH17 is also associated with I type gastric cancer. CDH17 encodes cadherin-17, also referred to as liver–intestine (LI)-cadherin, and is a structurally unique member of the cadherin superfamily [11]. A significant association was observed between CDH17 and CDX2 expression, as well as MUC2-positive I type gastric cancer. CDH17 is induced by CDX2 and also by EGFR activation in gastric cancer cells. HOXA10 is a member of the homeobox gene superfamily of transcription factors that contain a helix-turn-helix DNA-binding motif, and participates in myeloid cell differentiation and proliferation [25]. HOXA10 is also associated with MUC2-positive I type gastric cancers. The role of HOXA10 in stomach carcinogenesis remains unknown.

Olfactomedin 4 (OLFM4), also referred to as GW112 or human G-CSF clone-1 (hGC-1), was originally cloned from human myeloid cells and may facilitate apoptosis, tumor growth, and invasion [28,63,64]. In the non-neoplastic gastrointestinal tract, OLFM4 is expressed in tall columnar epithelial cells at the bottom of the intestinal crypt where the Lrg-5 (leucine-rich-repeat containing G-protein-coupled receptor 5)-positive stem cells are located [35,53]. Strong OLFM4 expression was detected in 60% of gastric cancers, and a significant association was found with the differentiated type of gastric cancer and Reg IV-negative tumors [35]. OLFM4 expression was more frequently observed in G type than in other types (I, GI and null). We have confirmed that combined measurement of Reg IV and OLFM4 protein levels in sera revealed a sensitivity of 57% for the detection of gastric cancer [35]. During the course of the search for novel tumor suppressor genes by SAGE data analysis, CLDN18 (encoding claudin-18) was identified to be preserved in G type gastric cancer [38]. Claudins comprising 24 members (claudins 1–24) are components of tight junction strands and are expressed in an organ-specific manner [51]. CLDN18 was first identified as a down-stream target of the T/EBP/NKX2.1 homeodomain transcription factor and has two variants: lung-specific variant 1 and stomach-specific variant 2 [32]. CLDN18 is expressed in the cell membrane of all epithelial cells of the normal gastric mucosa. The expression of CLDN18 is lost in MUC2-positive I type, while MUC5AC-positive G type preserves CLDN18 expression. It is necessary for a functional analysis to be performed; preservation of CLDN18 may participate in the genesis of G type gastric cancer.

Epigenetic regulation by microRNAs and its pathobiological role in gastric cancer

Various epigenetic alterations are involved in stomach carcinogenesis. Of these, DNA methylation associated with histone modification and chromatin remodeling to inactivate tumor suppressor genes has been intensively studied [60]. A recent focus of attention was the role of microRNA in cancer development and progression [8]. miRNA is a new class of small non-coding RNAs of 19–25 nucleotides which are cleaved from 60 to 110-nucleotide pre-miRNA precursors by RNase III Dicer [5]. Single-stranded miRNAs bind through partial sequence homology to the 3′ untranslated region of potentially hundreds of target genes and cause degradation of mRNAs and inhibition of translation. Over 30% of human genes are believed to be regulated by this mechanism. While initial studies suggested that miRNAs generally function as tumor suppressors, recent evidence has revealed that miRNAs possess either anti-tumorigenic or oncogenic properties depending on target genes [42]. In the last two years, more than 40 papers on miRNA in gastric cancer have been published. These include down-regulation of miR-9, miR-31, miR-141, miR-143, miR-145, and miR-433, as well as up-regulation of miR-34, miR-128, and miR-421 in gastric cancer.

Ueda et al. [52], in collaboration with us, studied the relationship between miRNA expression and progression and prognosis of gastric cancer using 182 Japanese gastric cancer samples. Using miRNA microarray analysis, 22 miRNAs were up-regulated and 13 were down-regulated in gastric cancer in comparison with corresponding non-neoplastic gastric tissue. Up-regulated miRNAs include miR-181d, miR-181a, miR-181c, miR-181b, miR-21, miR-25, miR-92, miR-93, miR-17-5p, miR-106a, miR-20b, miR-135a, miR-425, miR-106b, miR-20a, miR-19b, miR-224, miR-18a, miR-135b, miR-19a, miR-345, and miR-191, while down-regulated miRNAs include miR-148a, miR-148b, miR-375, miR-29b, miR-29c, miR-152, miR-218, miR-451, miR-30d, miR-30a, miR-30b, miR-30c, and miR-422b. In relation to the histological classification of gastric cancer, miR-105, miR-100, miR-125b, miR-199a, miR-99a, miR-143, miR-145, and miR-133a are up-regulated in the diffuse type, whereas miR-373, miR-498, miR-202, and miR-494 are up-regulated in the intestinal type. miR-125b, miR-199a, and miR-433 are the most important miRNAs involved in cancer progression. Low expression of let-7 g and miR-433 and high expression of miR-214 are independent unfavorable prognostic markers in

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multivariable analysis. We independently examined the expression profile of miRNAs by microarray and found that miR-200a, miR-200b, miR-200c, and miR-141 were down-regulated in diffuse type gastric cancer. It is recognized that epithelial–mesenchymal transition (EMT) is an important process to form diffuse histology and initiate metastasis by enhancing the motility of tumor cells. In transforming growth factor-beta (TGF-beta)-induced EMT in murine mammary epithelial cell system, members of the miR-200 family are repressed during EMT. Overexpression of miR-200 hinders EMT by enhancing E-cadherin expression through direct targeting of ZEB1 and ZEB2, which encode transcriptional repressors of E-cadherin [7,22]. Therefore, reduced expression of miR-200 family may participate in the genesis of diffuse histology of gastric cancer by reducing E-cadherin expression through ZEB1 and ZEB2. On the other hand, we have previously demonstrated that 40% of gastric cancers overexpress E2F-1, a master transcription factor for cell cycle control [44]. It has been recently reported in gastric cancer that E2F-1 up-regulates miR-106b, miR-93, and miR-25, a cluster of intronic miRNAs hosted in Mcm7 (Minichromosome maintenance 7) gene, while miR-106b and miR-93 control E2F-1 expression, establishing a negative feedback loop [37]. Furthermore, these miRNAs impair TGF-beta-dependent cell cycle arrest and apoptosis by inhibiting the synthesis of $p21^{WAF1/CIP1}$ and Bim (Bcl-2-interacting mediator of cell death). Therefore, this is one of the mechanisms for TGF-beta resistance frequently found in gastric cancer besides the abnormalities in TGF-beta receptor-signaling pathway.

It is known that miRNA genes are also transcriptionally regulated by DNA methylation and chromatin remodeling as in protein-coding genes [39]. In stomach carcinogenesis, a mucosal field with *H. pylori* infection is believed to be a condition predisposed to cancer development. Ando et al. [3] studied methylation levels of three miRNA genes (miR-124a-1, miR-124a-2, and miR-124a-3) in gastric mucosa with and without *H. pylori* infection from individuals with and without gastric cancer and in gastric cancer tissues. In gastric mucosa taken from healthy individuals, the methylation levels were significantly higher with *H. pylori* infection than without, while in gastric mucosa without *H. pylori* infection, the methylation levels were significantly higher in non-cancerous gastric mucosa taken from gastric cancer patients than in those from healthy individuals. The methylation levels in gastric cancers were highly variable. From these findings, they suggest that methylation-silencing of miRNA genes, in addition to that of protein-coding genes, contribute to the formation of field defect (predisposed mucosal field) to gastric cancer.

Genetic polymorphism and gastric cancer risk

Genetic factors, in addition to environmental factors, substantially contribute to the development and progression of gastric cancer. Gonzalez et al. [14] described that genetic susceptibility must be crucial in a variety of processes relevant to gastric carcinogenesis, including (1) the mucosal protection in the face of *H. pylori* infection and other carcinogens; (2) the inflammatory response, which conditions the maintenance, severity, and outcome of the *H. pylori* infection; (3) the functioning of carcinogen detoxification and antioxidant protection; (4) the intrinsic variability of DNA repair processes; and (5) cell proliferation ability. For instance, variants of IL-1beta (*IL1B*) and IL-1 receptor antagonist (*IL1RN*) genes, *IL1B* (-31 T genotype), and *IL1RN* IVS 86 bp VNTR (2/2 genotype) increase IL-1beta production and inhibit gastric acid secretion [12]. These are associated with an increased risk of chronic hypochlorhydric response to *H. pylori* infection and an increased gastric cancer risk. Hamajima et al. [16] reviewed genetic factors involved in the development of *H. pylori*-related gastric cancer. Genetic polymorphisms of tumor necrosis factor-alpha gene (TNF-A) and possibly quinone oxidoreductase 1 (NQO1) are also associated with *H. pylori* infection. Upon *H. pylori* infection in gastric epithelial cells, injected CagA is phosphorylated by src family kinases, interacts with src homology 2 domain-containing protein tyrosine phosphatase (SHP-2), and transduces signal to downstream molecules participating in atrophic gastritis [19]. Frequent G/A SNP in the intron 3 of the PTPN11 gene encoding SHP2 is associated with gastric atrophy monitored by serum pepsinogen I/II in the Asian population but not in Caucasians [15].

As to cancer-related genes and cancer risk, we have reported that single nucleotide polymorphism (SNP) in the transmembrane domain of the *HER-2*/c-*erb*B2 (655 Ile > Val, A > G) significantly affects gastric cancer risk [23]. SNP in the promoter regions of the *MMP-9* (-1562C/T) does not alter cancer susceptibility but is associated with tumor invasion, metastasis, or stage grouping [29]. Other polymorphisms of cancer-related genes reported to show a significant association with gastric cancer risk include cyclin D1, CDH1 (E-cadherin), EGFR, $p16^{INK4A}$, $p21^{WAF1/CIP1}$, etc. [12]. Recently, the Study Group of Millennium Genome Project for Cancer, including us [49], reported that genetic variation in prostate stem cell antigen (PSCA) is associated with susceptibility to diffuse type gastric cancer. A two-stage genome-wide association study in Japan has identified a significant association between an intronic SNP (rs2976392) in PSCA and diffuse type gastric cancer, but the association is far less significant with the intestinal type. PSCA is expressed in differentiating gastric epithelial cells and is frequently silenced in gastric cancer. PSCA inhibits cell proliferation *in vitro*. Substitution of C with the risk allele T at a SNP (rs2294008) in the first exon reduces transcriptional activity. The same risk allele was also associated with diffuse type gastric cancer in Korea.

In addition to affecting cancer risk, genetic polymorphisms are also associated with therapeutic efficacy and toxicity of anti-cancer drugs [55]. The genotype of CYP2C19 influences the eradication rate of *H. pylori*, and is useful to predict the success of the treatment [43]. An important implication regarding chemotherapy is that the genotype of UDP-glucuronosyltransferase 1A1 (UGT1A1) gene affects the severity of toxicity during irinotecan therapy [20].

Cancer stem cells in gastric cancer

Although initiation of cancer cell may occur at single cell levels, most of cancers consist of heterogeneous cancer cell populations mimicking the hierarchy of stem cell lineage. The cancer stem cell hypothesis is not entirely new, but only recently, actual features of cancer stem cells have been characterized by advanced technology [21]. The existence of cancer stem cells was first proven in acute myeloid leukemia in 1997 [6] and verified in a variety of solid tumors, such as cancers of the breast, brain, prostate, pancreas, etc. [21]. As few as 100 cancer stem cells can grow and form tumor in immunodeficient mice [46]. Cancer stem cells are now defined as "cells within a tumor that possess the capacity for self-renewal and that can cause the heterogeneous lineages of cancer cells that constitute the tumor" [9]. Another important aspect of cancer stem cells is the resistance to anti-tumor therapy. A variety of cancer stem cells express ATP-binding cassette family of transporter proteins to pump out multiple chemotherapeutic drugs.

As to gastric cancer, Haraguchi et al. [17] initially studied cancer stem cells by using a flow cytometry-based side population (SP) technique with Hoechst 33342 dye, and found that SP cells occupied 0.6–2.2% of gastric cancer cells. Because SP is highly enriched in stem cells, this is a useful tool for stem cell studies if specific cell surface markers are unknown. The SP cells express ATP-binding cassette, subfamily G, group 2 (ABCG2) and ATP-binding cassette, subfamily B, member 1 (ABCB1, also referred to as MDR-1 or P-glycoprotein) and show evidence of chemoresistance, self-renewal

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with the ability to differentiate progeny, and high tumorigenicity [13,17]. The SP cells of gastric cancer express a variety of adhesion molecules at high levels and possess a high potential for peritoneal metastasis [33].

Identification of a specific cell surface marker for cancer stem cells is important to study its biology and clinical implication. Many cell surface markers have been identified in cancers arising in individual organs: $CD34^{+}CD38^{-}$ for acute myeloid leukemia, $CD44^{+}CD24^{-/low}ESA$ (epithelial-specific antigen)$^{+}$ for breast cancer, $CD44^{+}CD24^{+}ESA^{+}$ for pancreas cancer, $CD44^{+}$integrin $\alpha2/\beta1^{+}$ for prostate cancer, $CD133^{+}$ for brain tumor and colorectal cancer, etc. [1,6,26,36,54]. In gastric cancer, although definitely specific markers have not been identified, CD44 surely marks cancer stem cell populations [47]. CD44-positive gastric cancer cells show stem cell properties of self-renewal and have the ability to raise CD-negative cells. CD44 knockdown results in reduced spheroid colony formation, which is characteristic of cancer stem cells, and less tumor production in immunodeficient mice. The SP cells of gastric cancer strongly express CD44, integrin $\alpha2$, $\alpha5$, $\beta3$, $\beta5$, and known stemness markers, such as Oct3/4 and Sox2 [33]. It has been reported that other potential cancer stem cell markers, such as CD24, CD133, CD166, stage-specific embryonic antigen-1 (SSEA-1), and SSEA-4, did not show any correlation with tumorigenicity [47].

Cancer stem cells share a self-renewal feature of normal tissue stem cells. Clark and Fuller [10] described that there are two ways for tumors containing a subpopulation of cancer stem cells. In the first, oncogenic mutations may inactivate the constraints on normal stem cell expansion, resulting in cancer stem cells originating from normal stem cells. The difference between normal stem cells and cancer stem cells is suggested to lie in their degree of dependence on the stem cell niche, a specialized microenvironment where stem cells reside [27]. In the second, oncogenic mutations may arise that allow for aberrant activation of the stem cell self-renewal regulatory machinery in transit-amplifying cells [10]. In the intestinal carcinogenesis, specific loss of adenomatous polyposis coli (APC) in long-lived intestinal stem cells marked by Lgr5 results in transformation and progressively growing neoplasia [4]. Regarding the stomach, it has been shown that stem cells marked by mitochondrial DNA mutations expand in normal mucosa and intestinal metaplasia, which gives an idea of how field cancerization develops [30]. However, whether or not gastric cancer stem cells arise from a normal stem cell or from transit-amplifying progenitor cell remains to be elucidated.

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References

[1] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 3983–3988.
[2] R. Almeida, E. Silva, F. Santos-Silva, D.G. Silberg, J. Wang, C. De Bolós, L. David, Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas, J. Pathol. 199 (2003) 36–40.
[3] T. Ando, T. Yoshida, S. Enomoto, K. Asada, M. Tatematsu, M. Ichinose, T. Sugiyama, T. Ushijima, DNA methylation of microRNA genes in gastric mucosa of gastric cancer patients: its possible involvement in the formation of epigenetic field defect, Int. J. Cancer 124 (2009) 2367–2374.
[4] N. Barker, R.A. Ridgway, J.H. van Es, M. van de Wetering, H. Begthel, M. van den Born, E. Danenberg, A.R. Clarke, O.J. Sansom, H. Clevers, Crypt stem cells as the cells-of-origin of intestinal cancer, Nature 457 (2009) 608–611.
[5] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[6] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, Nat. Med. 3 (1997) 730–737.
[7] C.P. Bracken, P.A. Gregory, N. Kolesnikoff, A.G. Bert, J. Wang, M.F. Shannon, G.J. Goodall, A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial–mesenchymal transition, Cancer Res. 68 (2008) 7846–7854.
[8] G.A. Calin, C.M. Croce, MicroRNA-cancer connection: the beginning of a new tale, Cancer Res. 66 (2006) 7390–7394.
[9] M.F. Clarke, J.E. Dick, P.B. Dirks, C.J. Eaves, C.H. Jamieson, D.L. Jones, J. Visvader, I.L. Weissman, G.M. Wahl, Cancer stem cells—perspectives on current status and future directions: AACR workshop on cancer stem cells, Cancer Res. 66 (2006) 9339–9344.
[10] M.F. Clarke, M. Fuller, Stem cells and cancer: two faces of eve, Cell 124 (2006) 1111–1115.
[11] A.H. Dantzig, J.A. Hoskins, L.B. Tabas, S. Bright, R.L. Shepard, I.L. Jenkins, D.C. Duckworth, J.R. Sportsman, D. Mackensen, P.R. Rosteck Jr., P.L. Skatrud, Association of intestinal peptide transport with a protein related to the cadherin superfamily, Science 264 (1994) 430–433.
[12] E.M. El-Omar, M. Carrington, W.H. Chow, K.E. McColl, J.H. Bream, H.A. Young, J. Herrera, J. Lissowska, C.C. Yuan, N. Rothman, G. Lanyon, M. Martin, J.F. Fraumeni Jr., C.S. Rabkin, Interleukin-1 polymorphisms associated with increased risk of gastric cancer, Nature 404 (2000) 398–402.
[13] K. Fukuda, Y. Saikawa, M. Ohashi, K. Kumagai, M. Kitajima, H. Okano, Y. Matsuzaki, Y. Kitagawa, Tumor initiating potential of side population cells in human gastric cancer, Int. J. Oncol. 34 (2009) 1201–1207.
[14] C.A. Gonzalez, N. Sala, G. Capella, Genetic susceptibility and gastric cancer risk, Int. J. Cancer 100 (2002) 249–260.
[15] Y. Goto, T. Ando, K. Yamamoto, A. Tamakoshi, E. El-Omar, H. Goto, N. Hamajima, Association between serum pepsinogens and polymorphism of PTPN11 encoding SHP-2 among *Helicobacter pylori* seropositive Japanese, Int. J. Cancer 118 (2006) 203–208.
[16] N. Hamajima, M. Naito, T. Kondo, Y. Goto, Genetic factors involved in the development of *Helicobacter pylori*-related gastric cancer, Cancer Sci. 97 (2006) 1129–1138.
[17] N. Haraguchi, T. Utsunomiya, H. Inoue, F. Tanaka, K. Mimori, G.F. Barnard, M. Mori, Characterization of a side population of cancer cells from human gastrointestinal system, Stem Cells. 24 (2006) 506–513.
[18] J.C. Hartupee, H. Zhang, M.F. Bonaldo, M.B. Soares, B.K. Dieckgraefe, Isolation and characterization of a cDNA encoding a novel member of the human regenerating protein family: Reg IV, Biochim. Biophys. Acta 1518 (2001) 287–293.
[19] M. Hatakeyama, Linking epithelial polarity and carcinogenesis by multitasking *Helicobacter pylori* virulence factor CagA, Oncogene 27 (2008) 7047–7054.
[20] F. Innocenti, S.D. Undevia, L. Iyer, P.X. Chen, S. Das, M. Kocherginsky, T. Karrison, L. Janisch, J. Ramírez, C.M. Rudin, E.E. Vokes, M.J. Ratain, Genetic variants in the *UDP-glucuronosyltransferase 1A1* gene predict the risk of severe neutropenia of irinotecan, J. Clin. Oncol. 22 (2004) 1382–1388.
[21] C.T. Jordan, M.L. Guzman, M. Noble, Cancer stem cells, N. Engl. J. Med. 355 (2006) 1253–1261.
[22] M. Korpal, E.S. Lee, Y. Kang, The miR-200 family inhibits epithelial–mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2, J. Biol. Chem. 283 (2008) 14910–14914.
[23] K. Kuraoka, N. Oue, S. Matsumura, Y. Hamai, R. Ito, H. Nakayama, W. Yasui, A single nucleotide polymorphism in the transmembrane domain coding region of HER-2 is associated with development and malignant phenotype of gastric cancer, Int. J. Cancer 107 (2003) 593–596.
[24] R. Kushima, W. Muller, M. Stolte, F. Borchad, Differential p53 protein expression in stomach adenomas of gastric and intestinal phenotypes: possible sequences of p53 alteration in stomach carcinogenesis, Virchows Arch. 428 (1996) 223–227.
[25] H.J. Lawrence, C. Largman, Homeobox genes in normal hematopoiesis and leukemia, Blood 80 (1992) 2445–2453.
[26] C. Li, D.G. Heidt, P. Dalerba, C.F. Burant, L. Zhang, V. Adsay, M. Wicha, M.F. Clarke, D.M. Simeone, Identification of pancreatic cancer stem cells, Cancer Res. 67 (2007) 1030–1037.
[27] L. Li, W.B. Neaves, Normal stem cells and cancer stem cells: the niche matters, Cancer Res. 66 (2006) 4553–4557.
[28] W. Liu, L. Chen, J. Zhu, G.P. Rodgers, The glycoprotein hGC-1 binds to cadherin and lectins, Exp. Cell Res. 312 (2006) 1785–1797.
[29] S. Matsumura, N. Oue, H. Nakayama, Y. Kitadai, K. Yoshida, Y. Yamaguchi, K. Imai, K. Nakachi, K. Matsusaki, K. Chayama, W. Yasui, A single nucleotide polymorphism of the MMP9 promoter affects tumor progression and invasive phenotype of gastric cancer, J. Cancer Res. Clin. Oncol. 131 (2005) 19–25.
[30] S.A. McDonald, L.C. Greaves, L. Gutierrez-Gonzalez, M. Rodriguez-Justo, M. Deheragoda, S.J. Leedham, R.W. Taylor, C.Y. Lee, S.L. Preston, M. Lovell, T. Hunt, G. Elia, D. Oukrif, R. Harrison, M.R. Novelli, I. Mitchell, D.L. Stoker, D.M. Turnbull, J.A. Jankowski, N.A. Wright, Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells, Gastroenterology 134 (2008) 500–510.
[31] Y. Mitani, N. Oue, S. Matsumura, K. Yoshida, T. Noguchi, M. Ito, S. Tanaka, H. Kuniyasu, N. Kamata, W. Yasui, Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy, Oncogene 26 (2007) 4383–4393.

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[32] T. Niimi, K. Nagashima, J.M. Ward, P. Minoo, D.B. Zimonjic, N.C. Popescu, S. Kimura, claudin-18, a novel downstream target gene for the T/EBP/NKX2.1 homeodomain transcription factor, encodes lung- and stomach-specific isoforms through alternative splicing, Mol. Cell. Biol. 21 (2001) 7380–7390.

[33] T. Nishii, M. Yashiro, O. Shinto, T. Sawada, M. Ohira, K. Hirakawa, Cancer stem cell-like SP cells have a high adhesion ability to the peritoneum in gastric carcinoma, Cancer Sci. 100 (2009) 1397–1402.

[34] N. Oue, Y. Hamai, Y. Mitani, S. Matsumura, Y. Oshimo, P.P. Aung, K. Kuraoka, H. Nakayama, W. Yasui, Gene expression profile of gastric carcinoma: identification of genes and tags potentially involved in invasion, metastasis, and carcinogenesis by serial analysis of gene expression, Cancer Res. 64 (2004) 2397–2405.

[35] N. Oue, K. Sentani, T. Noguchi, S. Ohara, N. Sakamoto, T. Hayashi, K. Anami, J. Motoshita, M. Ito, S. Tanaka, K. Yoshida, W. Yasui, Serum olfactomedin 4 (GW112, hGC-1) in combination with Reg IV is a highly sensitive biomarker for gastric cancer patients, Int. J. Cancer 125 (2009) 2383–2392.

[36] L. Patrawala, T. Calhoun-Davis, R. Schneider-Broussard, D.G. Tang, Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells, Cancer Res. 67 (2007) 6796–6805.

[37] F. Petrocca, R. Visone, M.R. Onelli, M.H. Shah, M.S. Nicoloso, I. de Martino, D. Iliopoulos, E. Pilozzi, C.G. Liu, M. Negrini, L. Cavazzini, S. Volinia, H. Alder, L.P. Ruco, G. Baldassarre, C.M. Croce, A.A. Vecchione, E2F1-regulated microRNAs impair TGFβ-dependent cell-cycle arrest and apoptosis in gastric cancer, Cancer Cell 13 (2008) 272–286.

[38] Y. Sanada, N. Oue, Y. Mitani, K. Yoshida, H. Nakayama, W. Yasui, Down-regulation of the claudin-18 gene, identified through serial analysis of gene expression data analysis, in gastric cancer with an intestinal phenotype, J. Pathol. 208 (2006) 633–642.

[39] G.K. Scott, M.D. Mattie, C.E. Berger, S.C. Benz, C.C. Benz, Rapit alteration of microRNA levels by histone deacetylase inhibition, Cancer Res. 66 (2006) 1277–1281.

[40] G.E. Schepers, R.D. Teasdale, P. Koopman, Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families, Dev. Cell 3 (2002) 167–170.

[41] N. Shibata, J. Watari, M. Fujiya, S. Tanno, Y. Saitoh, Y. Kohgo, Cell kinetics and genetic instabilities in differentiated type early gastric cancers with different mucin phenotype, Hum. Pathol. 34 (2003) 32–40.

[42] R. Spizzo, M.S. Nicoloso, C.M. Croce, G.A. Calin, SnapShot: microRNAs in cancer, Cell 137 (2009) 586.

[43] T. Suzuki, K. Matsuo, A. Sawaki, K. Wakai, K. Hirose, H. Ito, T. Saito, T. Nakamura, K. Yamao, N. Hamajima, K. Tajima, Influence of smoking and CYP2C19 genotype on *H. pylori* eradication success, Epidemiol. Infect. 135 (2007) 171–176.

[44] T. Suzuki, W. Yasui, H. Yokozaki, K. Naka, T. Ishikawa, E. Tahara, Expression of the E2F family in human gastrointestinal carcinomas, Int. J. Cancer 81 (1999) 535–538.

[45] Y. Tajima, T. Shimoda, Y. Nakanishi, N. Yokoyama, T. Tanaka, K. Shimizu, T. Saito, M. Kawamura, M. Kusano, K. Kumagai, Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions, Oncology 61 (2001) 212–220.

[46] S. Takaishi, T. Okumura, T.C. Wang, Gastric cancer stem cells, J. Clin. Oncol. 26 (2008) 2876–2882.

[47] S. Takaishi, T. Okumura, S. Tu, S.S. Wang, W. Shibata, R. Vigneshwaran, S.A. Gordon, Y. Shimada, T.C. Wang, Identification of gastric cancer stem cells using the cell surface marker CD44, Stem Cells 27 (2009) 1006–1020.

[48] M. Tatematsu, T. Tsukamoto, T. Mizoshita, History of gastric carcinoma research in Japan: basic aspects, in: M. Kaminishi, K. Takubo, K. Mafune (Eds.), The Diversity of Gastric Carcinoma, Springer-Verlag, Tokyo, 2005, pp. 3–28.

[49] The Study Group of Millennium Genome Project for Cancer, in: Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer, Nat. Genet. 40 (2008) 730–740.

[50] T. Tsukamoto, T. Mizoshita, M. Mihara, H. Tanaka, Y. Takenaka, Y. Yamamura, S. Nakamura, T. Ushijima, M. Tatematsu, Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes, Histopathology 46 (2005) 649–658.

[51] S. Tsukita, M. Furuse, M. Itoh, Multifunctional strands in tight junctions, Nat. Rev. Mol. Cell. Biol. 2 (2001) 285–293.

[52] T. Ueda, S. Volinia, H. Okumura, M. Shimizu, C. Taccioli, S. Rossi, H. Alder, C.-G. Liu, N. Oue, W. Yasui, K. Yoshida, H. Sasaki, S. Nomura, Y. Seto, M. Kaminishi, G. Calin, C.M. Carlo, Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis, Lancet Oncol. 11 (2010) 136–146.

[53] L.G. van der Flier, A. Haegebarth, D.E. Stange, M. van der Wetering, G. Clevers, OLFM4 is a robust marker for stem cell in human intestine and marks a subset of colorectal cancer cells, Gastroenterology 137 (2009) 15–17.

[54] L. Vermeulen, M. Todaro, F. de Sousa Mello, M.R. Sprick, K. Kemper, M. Perez Alea, D.J. Richel, G. Stassi, J.P. Medema, Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 13427–13432.

[55] J.W. Watters, H.L. McLeod, Cancer pharmacogenomics: current and future applications, Biochim. Biophys. Acta 1603 (2003) 99–111.

[56] W. Yasui, N. Oue, P.P. Aung, S. Matsumura, M. Shutoh, H. Nakayama, Molecular-pathological prognostic factors of gastric cancer: a review, Gastric Cancer 8 (2005) 86–94.

[57] W. Yasui, N. Oue, R. Ito, K. Kuraoka, H. Nakayama, Search for new biomarkers of gastric cancer through serial analysis of gene expression and its clinical implications, Cancer Sci. 95 (2004) 385–392.

[58] W. Yasui, N. Oue, Y. Kitadai, H. Nakayama, Recent advances in molecular pathobiology of gastric carcinoma, in: M. Kaminishi, K. Takubo, K. Mafune (Eds.), The Diversity of Gastric Carcinoma, Springer-Verlag, Tokyo, 2005, pp. 51–71.

[59] W. Yasui, N. Oue, H. Kuniyasu, R. Ito, E. Tahara, H. Yokozaki, Molecular diagnosis of gastric cancer: present and future, Gastric Cancer 4 (2001) 113–121.

[60] W. Yasui, N. Oue, S. Ono, Y. Mitani, R. Ito, H. Nakayama, Histone acetylation and gastrointestinal carcinogenesis, Ann. N. Y. Acad. Sci. 983 (2003) 220–231.

[61] W. Yasui, N. Oue, K. Sentani, N. Sakamoto, J. Motoshita, Transcriptome dissection of gastric cancer: identification of novel diagnostic and therapeutic targets from pathology specimens (review article), Pathol. Int. 59 (2009) 121–136.

[62] W. Yasui, K. Sentani, J. Motoshita, H. Nakayama, Molecular pathobiology of gastric cancer, Scand. J. Surg. 95 (2006) 225–231.

[63] X. Zhang, Q. Huang, Z. Yang, Y. Li, C.Y. Li, GW112, a novel antiapoptotic protein that promotes tumor growth, Cancer Res. 64 (2004) 2474–2481.

[64] J. Zhang, W.L. Liu, D.C. Tang, L. Chen, M. Wang, S.D. Pack, Z. Zhuang, G.P. Rodgers, Identification and characterization of a novel member of olfactomedin-related protein family, hGC-1, expressed during myeloid lineage development, Gene 283 (2002) 83–93.

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Desmocollin 2 is a new immunohistochemical marker indicative of squamous differentiation in urothelial carcinoma

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Desmocollin 2 is a new immunohistochemical marker indicative of squamous differentiation in urothelial carcinoma

Aims: Urothelial carcinoma (UC) with squamous differentiation tends to present at higher stages than pure UC. To distinguish UC with squamous differentiation from pure UC, a sensitive and specific marker is needed. Desmocollin 2 (DSC2) is a protein localized in desmosomal junctions of stratified epithelium, but little is known about its biological significance in bladder cancer. We examined the utility of DSC2 as a diagnostic marker.
Methods and results: We analysed the immunohistochemical characteristics of DSC2, and studied the relationship of DSC2 expression with the expression of the known markers uroplakin III (UPIII), cytokeratin (CK)7, CK20, epidermal growth factor receptor (EGFR), and p53. DSC2 staining was detected in 24 of 25 (96%) cases of UC with squamous differentiation, but in none of 85 (0%) cases of pure UC. DSC2 staining was detected only in areas of squamous differentiation. DSC2 expression was mutually exclusive of UPIII expression, and was correlated with EGFR expression. Furthermore, DSC2 expression was correlated with higher stage ($P = 0.0314$) and poor prognosis ($P = 0.0477$).
Conclusions: DSC2 staining offers high sensitivity (96%) and high specificity (100%) for the detection of squamous differentiation in UC. DSC2 is a useful immunohistochemical marker for separation of UC with squamous differentiation from pure UC.

Keywords: bladder cancer, desmocollin 2, epidermal growth factor receptor, prognosis, uroplakin III, urothelial carcinoma with squamous differentiation

Abbreviations: CK, cytokeratin; DSC, desmocollin; DSG, desmoglein; EGFR, epidermal growth factor receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RNAi, RNA interference; SCC, squamous cell carcinoma; siRNA, small interfering RNA; UC, urothelial carcinoma; UPIII, uroplakin III

Introduction

Urothelial carcinoma (UC) includes the majority of bladder cancers. However, UC has a propensity for divergent differentiation. UC with squamous differentiation, which is the most common mixed histological feature, occurs in 10–60% of UC cases.[1–4] Although the clinical significance of UC with squamous differentiation

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