Jpn. J. Cancer Res. **93**, 1114–1122, October 2002

Differentially Regulated Genes as Putative Targets of Amplifications at 20q in Ovarian Cancers

Takafumi Watanabe,[1,2] Issei Imoto,[1] Tomoyuki Katahira,[3] Akira Hirasawa,[1] Isamu Ishiwata,[4] Mitsuru Emi,[5] Masaomi Takayama,[6] Akira Sato[2] and Johji Inazawa[1,7]

[1]Department of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, [2]Department of Obstetrics and Gynecology, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, [3]Department of Obstetrics and Gynecology, National Nagoya Hospital, 4-1-1 San-no-maru, Naka-ku, Nagoya 460-0001, [4]Ishiwata Obstetrics and Gynecologic Hospital, 1-4-21 Kamimito, Mito 310-0041, [5]Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211-8533 and [6]Department of Obstetrics and Gynecology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402

Frequent amplification of DNA at 20q or part of 20q has been demonstrated by comparative genomic hybridization in ovarian cancer (OC), but the genetic target(s) of these amplification events remain unknown. We examined copy-number changes with respect to six candidate genes, *E2F1* (20q11.2), *TGIF2* (20q11.2), *AIB1* (20q12), *PTPN1* (20q13.1), *ZNF217* (20q13.2), and *BTAK* (20q13), and then measured transcription levels of each candidate in 18 OC cell lines. Three distinct cores of amplification were identified: 20q11.2, harboring *E2F1* and *TGIF2* (region I; 1 of 18 cell lines, 5.6%); 20q13.1, harboring *PTPN1* (region II; 5 lines, 27.8%); and 20q13.2, harboring *ZNF217* and *BTAK* (region III; 6 lines, 33.3%). Among the six genes examined, expression levels of *PTPN1* and *ZNF217* were significantly correlated with absolute copy-number, and those of *PTPN1* and *TGIF2* were significantly correlated with copy-number relative to the centromere of chromosome 20 (20cen). Among 19 primary OCs examined, moreover, we observed amplification of *TGIF2*, *PTPN1* and *ZNF217* in five (26.3%), ten (52.6%), and twelve (63.2%) tumors, respectively. Expression levels of *PTPN1* and *ZNF217* were significantly correlated with their copy-numbers in those primary OCs. Our results suggest that 20q amplifications in OCs can be extensive and complex, probably due to synergistic or non-synergistic amplification of separate regions of 20q, involving multiple, independently amplified targets.

Key words: 20q — Amplification — Ovarian cancer

Among a variety of genetic alterations that may occur during development and progression of ovarian cancer (OC), DNA amplification is of particular importance because it can activate specific genes that confer a selective growth advantage. The regions most frequently amplified in OC, 17q21, 8q24 and 12p11, contain the *ERBB2*, *MYC*, and *KRAS* genes, respectively.[1)] Recently, additional but less fully characterized amplicons have been identified by means of comparative genomic hybridization (CGH). Among them, 20q or part of 20q appears to be amplified in 20–50% of OCs.[2–5)] This region is often amplified in other types of tumor as well, including breast, colon, and pancreatic cancers,[6–8)] where amplification of 20q has been implicated in metastasis and poor prognosis.[8–10)] It is reasonable to suppose that 20q may harbor dominantly acting genetic elements that contribute to the progression of OC, as well as other types of tumors.

Levels and patterns of amplification at 20q appear to be highly various and complex. Three distinct non-syntenic regions, 20q11, 20q12-13.1, and 20q13.2, have been identified in breast cancers.[11)] Using array-CGH, Pinkel *et al.*[12)] also detected complex changes in copy-number and structure of the 20q region in breast cancers; that is, 20q seems to harbor more than two genes as targets for amplification. Although on functional grounds several candidates in the region, e.g. *E2F1* (20q11.2), *TGIF2* (20q11.2), *AIB1* (20q12), *PTPN1* (20q13.1), *ZNF217* (20q13.2), and *BTAK* (20q13.2), have been proposed as targets for the 20q amplification in breast cancer,[11,13–20)] targets in OC remain unknown. In order to reveal some of these targets, we examined the six genes in OC cell lines and primary tumors. To be defined as a target of an amplification event, a gene should be over-expressed and functionally activated via an amplification mechanism.[17,21,22)] Therefore we compared the copy-number status of each of the six candidates with their expression status in our OC materials.

MATERIALS AND METHODS

Cell lines and primary tumors Among the total of 18 OC cell lines examined, information about 14 of them

[7] To whom correspondence should be addressed.
E-mail: johinaz.cgen@mri.tmd.ac.jp

(HMOA, HUOA, HMKOA, HTOA, HIOA, W3UF, HTBOA, MCAS, Kuramochi, TYK-nu, TYK-nu.CP-r, RMG-I, RMG-II, and RMUG-L) has been published.[5] The other four lines (OVCAR3, OVCAR5, OVCAR8, and SKOV3) were purchased from American Type Culture Collection (ATCC). All lines were maintained in RPMI-1640 with 10% fetal calf serum and penicillin/strepto-mycin.

Nineteen unselected primary ovarian tumors (7 serous adenocarcinomas, 6 clear-cell carcinomas, 4 endometrioid adenocarcinomas, and 2 mucinous adenocarcinoma) were obtained from the Department of Obstetrics and Gynecology, National Nagoya Hospital or from Fukushima Medical University, with written consent from each patient in the formal style and after approval by the local ethics committees. All tumors were histologically classified with hematoxylin and eosin staining by one gynecological pathologist, and contained over 60% tumor cells.

Fluorescence *in situ* hybridization (FISH) Metaphase-chromosome slides were prepared from cell lines or normal peripheral blood lymphocytes for FISH experiments that were carried out in a standard manner.[23]

FISH analyses were performed using as probes biotin-16-dUTP- or digoxigenin-11-dUTP-labelled bacterial artificial chromosomes (BACs) or P1 artificial chromosomes (PACs) containing the six candidate genes, as follows: *E2F1* (RP11-607H21), *TGIF2* (RP4-277B1), *AIB1* (RP11-19D5), *PTPN1* (CITB-2022M04), *ZNF217* (RP4-724E16), and *BTAK* (GS-49G11). A PAC RP5-1059A9 specific for the centromere of chromosome 20 (20cen) was used as a reference.[24] Chromosomal *in situ* suppression hybridization and fluorescent detection of hybridization signals were carried out as described elsewhere.[23] The copy-number and molecular organization of the region of interest were assessed according to the hybridization patterns observed on both metaphase and interphase chromosomes. The copy-number of each locus was expressed as the absolute number of signals, or as the number of signals relative to the number of 20cen signals. Precise localization of each BAC was confirmed by FISH using normal metaphase chromosomes.

Northern blotting Ten-microgram aliquots of total RNA extracted from each cell line were electrophoresed in 1.0% agarose/0.67 *M* formaldehyde gel, and then transferred to a positively charged nylon membrane (Hybond N^+, Amersham Pharmacia Biotech, Tokyo). Probes for northern blotting consisted of Integrated Molecular Analysis of Genome and their Expression (IMAGE) cDNA clones 236142 (*E2F1*), 502333 (*AIB1*), 1560118 (*PTPN1*), 1360920 (*ZNF217*), and 1137958 (*BTAK*) purchased from Incyte Genomic, Inc. (St. Louis, MO); in addition, we used a full-length cDNA clone of *TGIF2*,[18] and a control probe (glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*). All probes were labeled with [α-^{32}P]dCTP and hybridized to pre-hybridized blots. The hybridized blots were washed and exposed as described elsewhere.[22] Autoradiographic signals were analyzed using Mac Bas (Fuji Film, Tokyo); each signal was normalized by reference to the value obtained for *GAPDH* and reported as a normalized expression level.[21]

Real-time quantitative polymerase chain reaction (PCR) and reverse transcriptase-PCR (RT-PCR) The quantification of genomic DNA and messenger RNA (mRNA) levels of genes in primary tumors was carried out using a real-time fluorescence detection method.[25–27] Single-stranded complementary DNA (cDNA) was generated from total RNA using "SuperScript" First-Strand Synthesis System (Invitrogen, Carlsbad, CA) following the manufacturer's directions. Real-time quantitative PCR was performed using a LightCycler (Roche Diagnostics, Tokyo) with CYBR Green according to the manufacturer's protocol. β_2-Microglobulin (*B2M*) and *GAPDH* were used as the endogenous controls for genomic and mRNA levels, respectively, and genomic DNA copy-number and expression level of each sample were normalized on the basis of *B2M* and *GAPDH* levels, respectively. Primer sequences for each gene are available on request. Duplicate PCR amplification was performed for each sample.

Statistical analysis The relationship between copy-number or relative copy-number and the normalized expression level or relative copy-number of each gene was calculated using Spearman's test, and correlation coefficients and associated probability (*P*) were calculated. $P<0.05$ was required for significance.

RESULTS

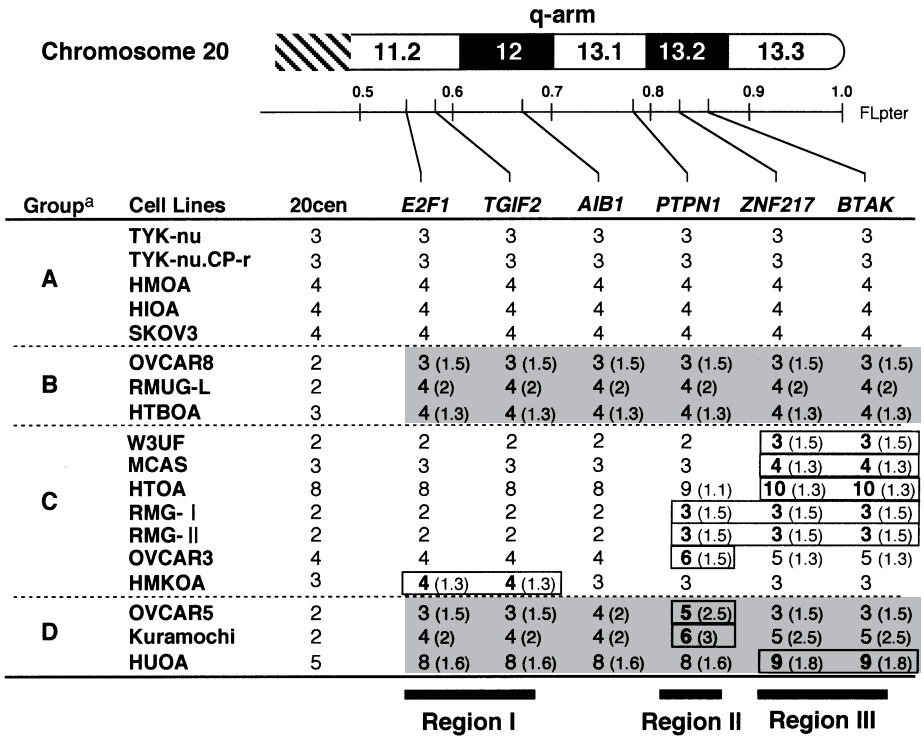
Amplification status at 20q in OC cell lines We first evaluated the copy-number status of the six positional and functional candidate genes by FISH in 18 OC cell lines (Fig. 1). CGH analysis had been carried out previously in 14 of these lines,[5] and gain of DNA on 20q had been detected in eight of them (57.1%). By FISH, however, copy-number increases in 20q or part of 20q were detected in all lines. As shown in Figs. 1 and 2, four patterns of DNA increase along 20q were found: group A, gain of chromosome 20; group B, gain of the whole q-arm of chromosome 20 (whole-20q gain); group C, gain of specific loci (fewer than 5 of the 6 loci) without whole-20q gain; and group D, gain of specific loci with whole-20q gain. We defined as "cores of amplification" regions containing the loci with increased copy-numbers in groups C and D (Fig. 1). When the prevalence of the core of amplification was evaluated, we found three distinct regions along 20q, i.e. 20q11.2, harboring *E2F1* and *TGIF2* (region I; 1 of 18 cell lines, 5.6%); 20q13.1, harboring *PTPN1* (region II; 5/18, 27.8%); and 20q13.2, harboring *ZNF217* and *BTAK* (region III; 6/18, 33.3%) (Fig. 1). The cores of amplification in cell lines RMG-I and RMG-II (2/

18, 11.1%) spanned region II and region III. Among the eight cell lines of groups A and B (44.4%) we detected no cores of amplification (Fig. 1), although gain of the whole q-arm had been observed in cell lines of group B.

Comparison of DNA copy-numbers or relative copy-numbers with expression levels of candidate genes in OC cell lines To determine whether amplification of candidate targets in 20q was consistently associated with their expression levels, we examined transcription of the six genes in question. Fig. 2 shows images from northern-blot and FISH analyses of *ZNF217* and *PTPN1* in representative cell lines. Levels of *ZNF217* expression in cell lines with increased copy-numbers (HTOA and HUOA) were higher than in cell lines with low copy-numbers (HMKOA and RMG1). Results in the same cell lines were similar for *PTPN1* (data not shown). We were able to validate a significant and positive correlation between expression level and copy-number only for *PTPN1* and *ZNF217*, indicating that those two genes are consistently over-expressed in some OCs in a gene dosage-dependent manner (Fig. 3A). Non-significant but weak correlations were observed for *AIB1* (P=0.057) and *TGIF2* (P=0.069), but the copy-number of *BTAK* did not correlate with its expression level at all (P=0.676).

On the other hand, levels of *PTPN1* expression in cell lines showing relative increases in copy-number (Kuramochi and OVCAR5) were much higher than in cell lines without relative increases (HMOA and W3UF; Fig. 2B). We had observed the same result with *TGIF2* in earlier experiments.[18] We observed significant and positive correlation between expression levels and relative copy-numbers only for *PTPN1* and *TGIF2*; thus, these two genes were consistently over-expressed through an increase in DNA relative to 20cen (Fig. 3B). No significant correlation between these parameters was observed for the other four genes.

Amplification and expression status of potential target genes in primary tumors of OC Since *TGIF2*, *PTPN1*,



Group[a]	Cell Lines	20cen	*E2F1*	*TGIF2*	*AIB1*	*PTPN1*	*ZNF217*	*BTAK*
A	TYK-nu	3	3	3	3	3	3	3
	TYK-nu.CP-r	3	3	3	3	3	3	3
	HMOA	4	4	4	4	4	4	4
	HIOA	4	4	4	4	4	4	4
	SKOV3	4	4	4	4	4	4	4
B	OVCAR8	2	**3** (1.5)	**3** (1.5)	**3** (1.5)	**3** (1.5)	**3** (1.5)	**3** (1.5)
	RMUG-L	2	**4** (2)	**4** (2)	**4** (2)	**4** (2)	**4** (2)	**4** (2)
	HTBOA	3	**4** (1.3)	**4** (1.3)	**4** (1.3)	**4** (1.3)	**4** (1.3)	**4** (1.3)
C	W3UF	2	2	2	2	2	**3** (1.5)	**3** (1.5)
	MCAS	3	3	3	3	3	**4** (1.3)	**4** (1.3)
	HTOA	8	8	8	8	9 (1.1)	**10** (1.3)	**10** (1.3)
	RMG-I	2	2	2	2	**3** (1.5)	**3** (1.5)	**3** (1.5)
	RMG-II	2	2	2	2	**3** (1.5)	**3** (1.5)	**3** (1.5)
	OVCAR3	4	4	4	4	**6** (1.5)	5 (1.3)	5 (1.3)
	HMKOA	3	**4** (1.3)	**4** (1.3)	3	3	3	3
D	OVCAR5	2	**3** (1.5)	**3** (1.5)	**4** (2)	**5** (2.5)	**3** (1.5)	**3** (1.5)
	Kuramochi	2	**4** (2)	**4** (2)	**4** (2)	**6** (3)	**5** (2.5)	**5** (2.5)
	HUOA	5	**8** (1.6)	**8** (1.6)	**8** (1.6)	**8** (1.6)	**9** (1.8)	**9** (1.8)
			Region I			Region II	Region III	

Fig. 1. DNA copy-numbers of six different probes along 20q in 18 OC cell lines, with a 20cen probe as a reference, as determined by FISH. A chromosome ideogram and the FLpter scale are given to indicate the location of each probe (see ref. 19). FISH signals were measured in both metaphase and interphase chromosomes of each cell line; when signals were <1, relative copy-numbers are shown in parentheses. Copy-number changes indicating gain of the whole q-arm of chromosome 20 (whole 20q gain) are shaded; copy-number changes indicating the core of 20q-amplification are enclosed by rectangles. When the prevalence of each core of amplification was evaluated, three distinct regions became apparent: 20q11.2, harboring *E2F1* and *TGIF2* (region I); 20q13.1, harboring *PTPN1* (region II); and 20q13.2, harboring *ZNF217* and *BTAK* (region III). [a]Four patterns of DNA copy-number increase along 20q; A, gain of chromosome 20; B, gain of whole 20q; C, gain of specific loci without whole-20q gain; and D, gain of specific loci with whole-20q gain.

and *ZNF217* were present in the "core of amplification" and their expression levels correlated with either the absolute copy-number or the copy-number relative to 20cen, or both, we analyzed copy-number and expression status of these three genes in 19 primary OCs. Since limited amounts of DNA and RNA were available from primary tumors, we determined copy-number and expression level of each gene using a real-time quantitative PCR.[25–27] As shown in Fig. 4A, clear amplifications of *TGIF2*, *PTPN1*, and *ZNF217* were observed in five (26.3%), ten (52.6%), and twelve (63.2%) tumors, respectively, if amplification was defined as the ratio of each gene/*B2M* greater than 2. Interestingly, a statistically significant correlation was observed between copy-number of *PTPN1* and *ZNF217*, suggesting that co-amplification of these two genes may frequently occur in primary OCs (Fig. 4B). In addition, relative expression levels of *PTPN1* and *ZNF217* were significantly correlated with their copy-numbers normalized on the basis of *B2M* (P=0.0174 and 0.0087, respectively; Fig. 4C), while the relative expression level of *TGIF2* was of marginal significance (P=0.0683; data not shown).

DISCUSSION

Although amplification at 20q or part of 20q has been demonstrated by CGH in a number of ovarian tumors,[2–5] the target genes for the amplification event(s) remain unknown. As in other types of tumors such as breast cancer, gain or amplification at 20q appears variable in OC and many genes that could be targets of amplification events are present in the region. In the present study, therefore, we first examined the change in copy-numbers of six genes that emerged as likely candidates from a survey of the literature covering a relatively large series of OC cell lines, and we identified three distinct cores of amplification. However, since genomic analysis alone did not enable us to evaluate the roles of these genes in the tumorigenesis of OC, we measured transcription levels of these candidates in 18 OC cell lines and a panel of primary tumors. A similar strategy has guided studies to explore target genes for relatively large amplified regions, such as 17q22-23 in breast cancer[28–31] or 3q26 in several other types of cancer.[21, 32–34]

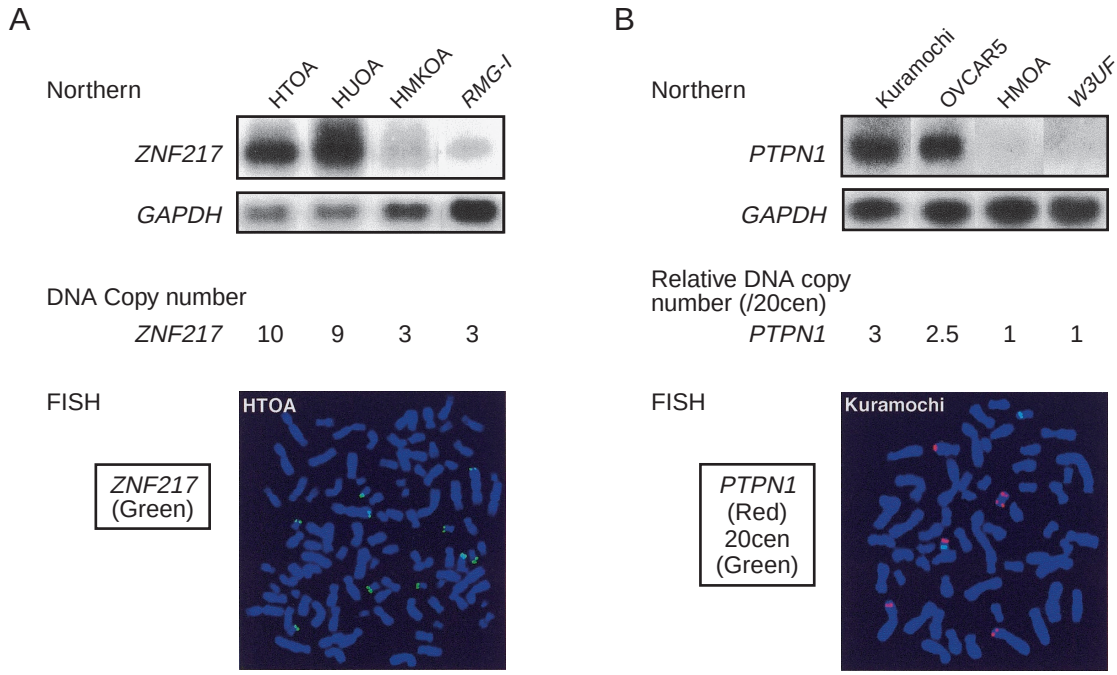


Fig. 2. (A) Upper: northern-blot analysis of *ZNF217* in four representative OC cell lines. Middle: DNA copy-number at the *ZNF217* locus as determined by FISH (see Fig. 1). Lower: representative FISH image from the *ZNF217*-specific probe in the HTOA cell line. The two cell lines (HTOA and HUOA) having higher copy-numbers of *ZNF217* clearly showed higher expression of this gene than two other cell lines (HMKOA and RMG1) having lower copy-numbers. (B) Upper: representative result of northern-blot analysis of *PTPN1* in four different OC cell lines. Middle: relative DNA copy-numbers (/20p) at the *PTPN1* locus determined by FISH analysis. Lower: representative image of two-color FISH analysis using a *PTPN1*-specific probe (red) and a 20cen probe (green) in the Kuramochi cell line. Cell lines Kuramochi and OVCAR5, with higher relative copy-numbers of *PTPN1* clearly showed higher expression of this gene than two cell lines (HMOA and W3UF) with lower relative copy-numbers.

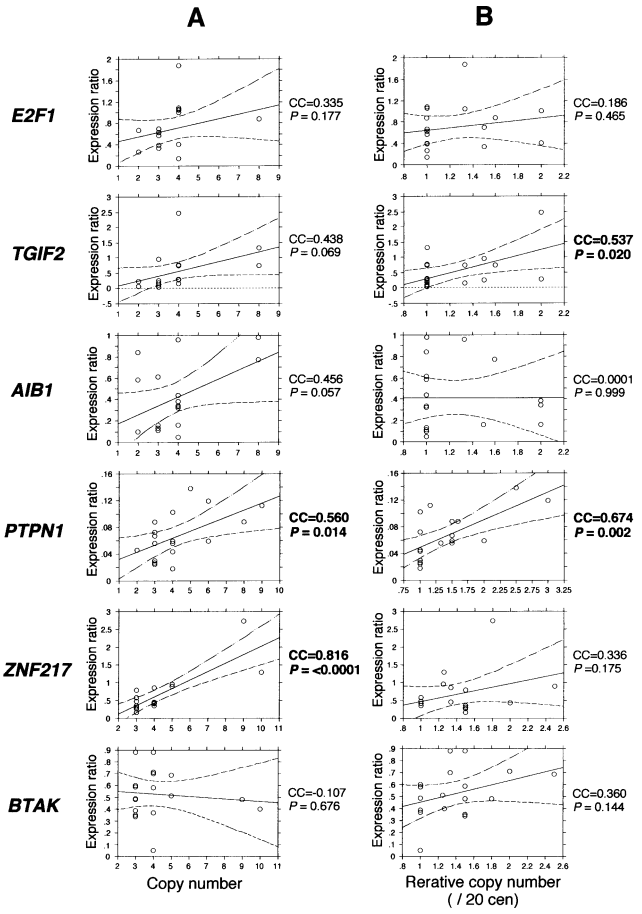


Fig. 3. Correlation between copy-number (A) or relative copy-number (B) and expression level of each gene in 18 OC cell lines. Copy-numbers (*x* axes) were obtained from FISH analyses; relative copy-numbers were calculated by dividing the copy-number of each gene by that of the 20cen reference (see Fig. 1). Normalized expression levels (*y* axes) were calculated from the intensity of each signal on a northern blot, divided by the corresponding reference value obtained from *GAPDH* expression (see ref. 21). Significant results ($P<0.05$) are shown in boldface type. CC: correlation coefficient.

Although amplified chromosomal regions usually span a region of several Mb, which consists of several amplification units and harbors many different genes, the number of target genes activated via amplification is usually limited.[22, 27, 35, 36] Despite amplification, several genes in the amplicon are not up-regulated, even though they have previously been nominated as candidate targets for amplification or are known to be oncogenes in specific types of tumors,[35, 36] and the reasons for that have not been elucidated yet. Two criteria are necessary for defining an amplification target in tumors of interest: (a) the putative target gene is within the core of the amplification, and (b) amplification consistently leads to over-expression of the gene.[17, 21, 22] Using these criteria we attempted to determine possible targets within the 20q amplicon in OCs, and identified significant correlations between expression levels and absolute or relative copy-numbers of *TGIF2*, *PTPN1*, and *ZNF217*. Each of these three genes was located in a separate core of amplification. Their amplification and increased expression dependent on DNA copy-number were also observed in primary OC tumors, although the relation between copy-number and expression level of *TGIF2* was of marginal significance.

Among the six genes examined, expression levels of *PTPN1* and *ZNF217* were significantly correlated with absolute copy-number, whereas those of *PTPN1* and *TGIF2* were significantly correlated with copy-numbers relative to 20cen. Amplification and concomitant increases in expression of candidate genes are strong evidence for identifying targets for amplification that may be associated with development and/or progression of tumors. It remains unclear whether any biological differences exist between the increases in absolute as opposed to relative copy-numbers. However, increases in relative copy-number of 20q13.2 in colorectal cancer, and of *MYC* in prostate cancer, have been correlated with metastatic potential and poor survival outcomes, respectively.[37, 38] Those findings suggest that not only numerical aberrations of DNA segments, but also a change in copy-number of specific regions on chromosomes, are associated with some tumor phenotypes. Genes undergoing gains in copy-number might be structurally or functionally affected by nearby chromosomal regions, or even by regions distant from them.

ZNF217 encodes a Krüppel-like zinc-finger transcription factor; this gene was originally identified based on its core location in the 20q13.2-amplicon observed in some breast cancers.[17] In colorectal cancers, amplification of *ZNF217* has been associated with increased metastatic potential.[38] Constitutively aberrant expression of *ZNF217* in human mammary epithelial cells promotes immortalization by overcoming senescence, increasing telomerase activity, and resisting inhibition of growth by transforming growth factor-beta (TGFβ).[39] In the present study we demonstrated that *ZNF217* is present in the region of 20q most frequently amplified among ovarian-cancer cell lines (region III, Fig. 1), and was concomitantly over-expressed in cells containing the relevant amplicon. All of these data together suggest that *ZNF217* may be a strong candidate for oncogenesis in 20q-amplified OCs.

We recently identified *TGIF2* as a novel TALE-superclass homeobox gene and a possible target for 20q-amplification in OC cell lines.[18] According to Melhuish *et al.*,[40] *TGIF2*, like *TGIF*, interacts with Smad3 to repress TGFβ-responsive transcription. We suggest that increased expres-

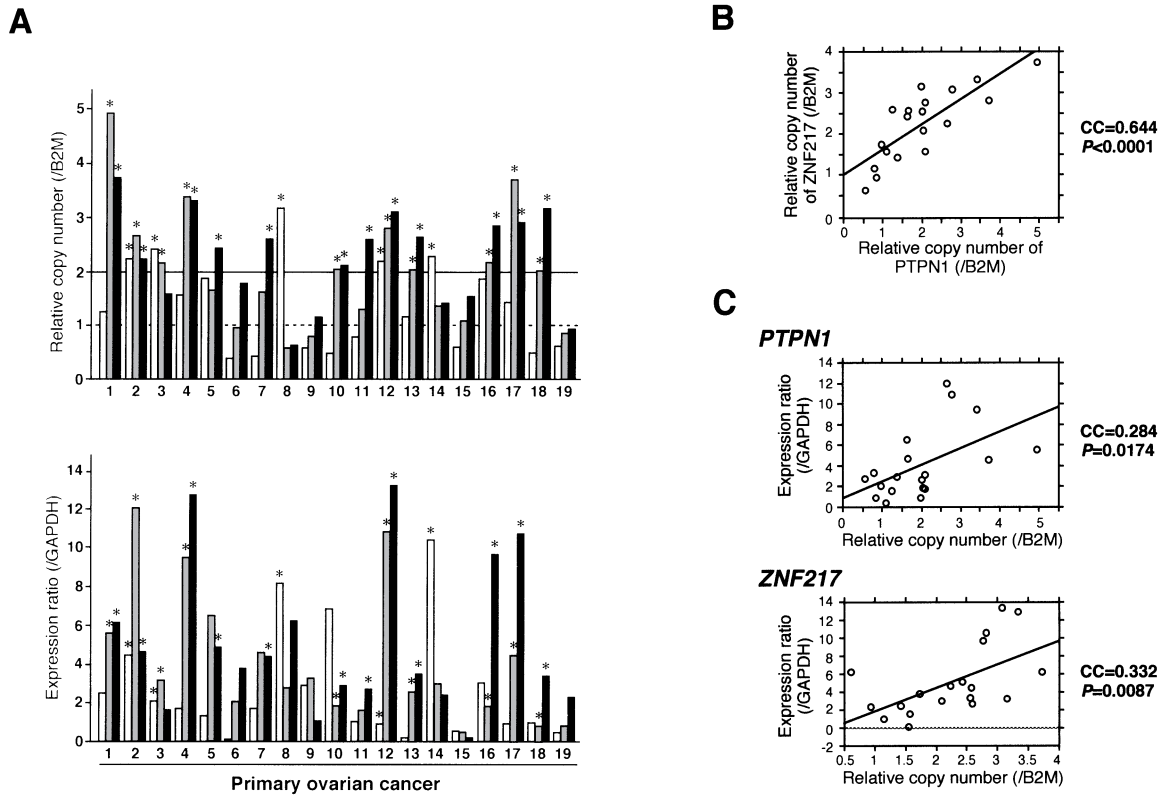


Fig. 4. Amplification and expression status of *TGIF2*, *PTPN1*, and *ZNF217* in 19 primary OCs. (A) Genomic copy-number ratios (upper) and relative expression levels (lower) of *TGIF2*, *PTPN1*, and *ZNF217* normalized on the basis of *B2M* and *GAPDH*, respectively. Asterisks indicate the cases showing the clear increase in copy-number (>2.0 relative to *B2M*) of each gene. □ *TGIF2*, ▩ *PTPN1*, ■ *ZNF217*. (B) A statistically significant correlation was observed between copy-number of *PTPN1* and *ZNF217* relative to *B2M*, suggesting that co-amplification of these two genes may occur frequently in primary OCs. (C) In 19 primary OCs, a statistically significant correlation between copy-number and relative expression level of candidate gene normalized on the basis of *B2M* and *GAPDH*, respectively, was observed in *PTPN1* and *ZNF217*. Significant results ($P<0.05$) are shown in boldface type. CC: correlation coefficient.

sion of *TGIF2* may play a role in tumor progression by decreasing the ability of TGFβ signals to control transcription of cell-cycle regulators.

PTPN1, encoding a non-receptor tyrosine phosphatase, is often over-expressed in breast and ovarian cancers.[11, 41, 42] In contrast to our results, Tanner *et al.*[11] found no correlation between amplification and expression of *PTPN1* in breast cancers. It is possible that expression of *PTPN1* might depend more strictly on its copy-number in ovarian tissues than in breast tissues.

We observed no significant correlation between expression level and absolute or relative copy-number for two outstanding candidates, *AIB1* and *BTAK*. *AIB1*, encoding a member of the SRC-1 family of nuclear-receptor coactivators, is amplified and over-expressed in many types of tumors including breast and ovarian cancers.[15] *AIB1* interacts with estrogen receptors in a ligand-dependent manner, enhancing estrogen-dependent transcription.[15] The fact that over-expression of *AIB1* does not always coincide with its amplification in breast and gastric cancers is consistent with our results.[15, 43] *BTAK*, encoding a centrosome-associated kinase, is involved in abnormalities of centrosome duplication-distribution in mammalian cells; up-regulation of this gene leads to amplification of centrosomes, resulting in chromosomal instability and aneuploidy.[44] Amplification and over-expression of *BTAK* have been detected in several cancer types, but its over-expression was not related to amplification in those experiments.[44, 45] Expression of *AIB1* and *BTAK*, therefore, is likely to be regulated by mechanisms other than amplification, such as activation of transcriptional machinery.

Amplifications of 20q in OCs appear to be extensive and complex, probably as a result of synergistic or non-synergistic amplifications of separate regions involving

multiple, independently amplified targets. Therefore, genes other than those we examined here could be activated as well, and contribute to development or progression of OC. Moreover, different combinations of targets may be associated with different malignant phenotypes. In order to understand the contribution of 20q amplification to ovarian carcinogenesis, other targets must be determined and, if co-amplified, examined as to their functional and/or structural correlation with other genes in the amplicons.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (B) and (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

(Received May 15, 2002/Revised July 16, 2002/Accepted July 19, 2002)

REFERENCES

1) Pejovic, T. Genetic changes in ovarian cancer. *Ann. Med.*, **27**, 73–78 (1995).
2) Iwabuchi, H., Sakamoto, M., Sakunaga, H., Ma, Y. Y., Carcangiu, M. L., Pinkel, D., Yang-Feng, T. L. and Gray, J. W. Genetic analysis of benign, low-grade, and high-grade ovarian tumors. *Cancer Res.*, **55**, 6172–6180 (1995).
3) Arnold, N., Hagele, L., Walz, L., Schempp, W., Pfisterer, J., Bauknecht, T. and Kiechle, M. Overrepresentation of 3q and 8q material and loss of 18q material are recurrent findings in advanced human ovarian cancer. *Genes Chromosom. Cancer*, **16**, 46–54 (1996).
4) Sonoda, G., Palazzo, J., du Manoir, S., Godwin, A. K., Feder, M., Yakushiji, M. and Testa, J. R. Comparative genomic hybridization detects frequent overrepresentation of chromosomal material from 3q26, 8q24, and 20q13 in human ovarian carcinomas. *Genes Chromosom. Cancer*, **20**, 320–328 (1997).
5) Watanabe, T., Imoto, I., Kosugi, Y., Ishiwata, I., Inoue, S., Takayama, M., Sato, A. and Inazawa, J. A novel amplification at 17q21-23 in ovarian cancer cell lines detected by comparative genomic hybridization. *Gynecol. Oncol.*, **81**, 172–177 (2001).
6) Kallioniemi, A., Kallioniemi, O. P., Piper, J., Tanner, M., Stokke, T., Chen, L., Smith, H. S., Pinkel, D., Gray, J. W. and Waldman, F. M. Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc. Natl. Acad. Sci. USA*, **91**, 2156–2160 (1994).
7) Ried, T., Knutzen, R., Steinbeck, R., Blegen, H., Schrock, E., Heselmeyer, K., du Manoir, S. and Auer, G. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. *Genes Chromosom. Cancer*, **15**, 234–245 (1996).
8) Ghadimi, B. M., Schrock, E., Walker, R. L., Wangsa, D., Jauho, A., Meltzer, P. S. and Ried, T. Specific chromosomal aberrations and amplification of the AIB1 nuclear receptor coactivator gene in pancreatic carcinomas. *Am. J. Pathol.*, **154**, 525–536 (1999).
9) Tanner, M. M., Tirkkonen, M., Kallioniemi, A., Holli, K., Collins, C., Kowbel, D., Gray, J. W., Kallioniemi, O. P. and Isola, J. Amplification of chromosomal region 20q13 in invasive breast cancer: prognostic implications. *Clin. Cancer Res.*, **1**, 1455–1461 (1995).
10) Korn, W. M., Yasutake, T., Kuo, W. L., Warren, R. S., Collins, C., Tomita, M., Gray, J. and Waldman, F. M. Chromosome arm 20q gains and other genomic alterations in colorectal cancer metastatic to liver, as analyzed by comparative genomic hybridization and fluorescence *in situ* hybridization. *Genes Chromosom. Cancer*, **25**, 82–90 (1999).
11) Tanner, M. M., Grenman, S., Koul, A., Johannsson, O., Meltzer, P., Pejovic, T., Borg, A. and Isola, J. J. Independent amplification and frequent co-amplification of three nonsyntenic regions on the long arm of chromosome 20 in human breast cancer. *Cancer Res.*, **56**, 3441–3445 (1996).
12) Pinkel, D., Segraves, R., Sudar, D., Clark, S., Poole, I., Kowbel, D., Collins, C., Kuo, W. L., Chen, C., Zhai, Y., Dairkee, S. H., Ljung, B. M., Gray, J. W. and Albertson, D. G. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat. Genet.*, **20**, 207–211 (1998).
13) Saito, M., Helin, K., Valentine, M. B., Griffith, B. B., Willman, C. L., Harlow, E. and Look, A. T. Amplification of the E2F1 transcription factor gene in the HEL erythroleukemia. *Genomics*, **25**, 130–138 (1995).
14) Guan, X. Y., Xu, J., Anzick, S. L., Zhang, H., Trent, J. M. and Meltzer, P. S. Hybrid selection of transcribed sequences from microdissected DNA: isolation of genes within amplified region at 20q11-q13.2 in breast cancer. *Cancer Res.*, **56**, 3446–3450 (1996).
15) Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M. and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science*, **277**, 965–968 (1997).
16) Sen, S., Zhou, H. and White, R. A. A putative serine/threonine kinase encoding gene BTAK on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. *Oncogene*, **14**, 2195–2200 (1997).
17) Collins, C., Rommens, J. M., Kowbel, D., Godfrey, T., Tanner, M., Hwang, S. I., Polikoff, D., Nonet, G., Cochran, J., Myambo, K., Jay, K. E., Froula, J., Cloutier, T., Kuo, W. L., Yaswen, P., Dairkee, S., Giovanola, J., Hutchinson, G. B., Isola, J., Kallioniemi, O. P., Palazzolo, M., Martin, C.,

Ericsson, C., Pinkel, D., Albertson, D., Li, W. B. and Gray, J. W. Positional cloning of ZNF217 and NABC1: genes amplified at 20q13.2 and overexpressed in breast carcinoma. *Proc. Natl. Acad. Sci. USA*, **95**, 8703–8708 (1998).

18) Imoto, I., Pimkhaokham, A., Watanabe, T., Saito-Ohara, F., Soeda, E. and Inazawa, J. Amplification and overexpression of TGIF2, a novel homeobox gene of the TALE superclass, in ovarian cancer cell lines. *Biochem. Biophys. Res. Commun.*, **276**, 264–270 (2000).
19) Tanner, M. M., Grenman, S., Koul, A., Johannsson, O., Meltzer, P., Pejovic, T., Borg, A. and Isola, J. J. Frequent amplification of chromosomal region 20q12-q13 in ovarian cancer. *Clin. Cancer Res.*, **6**, 1833–1839 (2000).
20) Collins, C., Volik, S., Kowbel, D., Ginzinger, D., Ylstra, B., Cloutier, T., Hawkins, T., Predki, P., Martin, C., Wernick, M., Kuo, W. L., Alberts, A. and Gray, J. W. Comprehensive genome sequence analysis of a breast cancer amplicon. *Genome Res.*, **11**, 1034–1042 (2001).
21) Imoto, I., Pimkhaokham, A., Fukuda, Y., Yang, Z. Q., Shimada, Y., Nomura, N., Hirai, H., Imamura, M. and Inazawa, J. SNO is a probable target for gene amplification at 3q26 in squamous-cell carcinomas of the esophagus. *Biochem. Biophys. Res. Commun.*, **286**, 559–565 (2001).
22) Imoto, I., Yang, Z. Q., Pimkhaokham, A., Tsuda, H., Shimada, Y., Imamura, M., Ohki, M. and Inazawa, J. Identification of cIAP1 as a candidate target gene within an amplicon at 11q22 in esophageal squamous cell carcinomas. *Cancer Res.*, **61**, 6629–6634 (2001).
23) Inazawa, J., Saito, H., Ariyama, T., Abe, T. and Nakamura, Y. High-resolution cytogenetic mapping of 342 new cosmid markers including 43 RFLP markers on human chromosome 17 by fluorescence *in situ* hybridization. *Genomics*, **17**, 153–162 (1993).
24) Bassi, C., Magnani, I., Sacchi, N., Saccone, S., Ventura, A., Rocchi, M., Marozzi, A., Ginelli, E. and Meneveri, R. Molecular structure and evolution of DNA sequences located at the alpha satellite boundary of chromosome 20. *Gene*, **256**, 43–50 (2000).
25) Wittwer, C. T., Herrmann, M. G., Moss, A. A. and Rasmussen, R. P. Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques*, **22**, 130–138 (1997).
26) Goff, L. K., Neat, M. J., Crawley, C. R., Jones, L., Jones, E., Lister, T. A. and Gupta, R. K. The use of real-time quantitative polymerase chain reaction and comparative genomic hybridization to identify amplification of the REL gene in follicular lymphoma. *Br. J. Haematol.*, **111**, 618–625 (2000).
27) Yasui, K., Arii, S., Zhao, C., Imoto, I., Ueda, M., Nagai, H., Emi, M. and Inazawa, J. TFDP1, CUL4A, and CDC16 identified as targets for amplification at 13q34 in hepatocellular carcinomas. *Hepatology*, **35**, 1476–1484 (2002).
28) Barlund, M., Monni, O., Kononen, J., Cornelison, R., Torhorst, J., Sauter, G., Kallioniemi, O. P. and Kallioniemi, A. Multiple genes at 17q23 undergo amplification and overexpression in breast cancer. *Cancer Res.*, **60**, 5340–5344 (2000).
29) Wu, G. J., Sinclair, C. S., Paape, J., Ingle, J. N., Roche, P. C., James, C. D. and Couch, F. J. 17q23 amplifications in breast cancer involve the PAT1, RAD51C, PS6K, and SIGma1B genes. *Cancer Res.*, **60**, 5371–5375 (2000).
30) Monni, O., Barlund, M., Mousses, S., Kononen, J., Sauter, G., Heiskanen, M., Paavola, P., Avela, K., Chen, Y., Bittner, M. L. and Kallioniemi, A. Comprehensive copy number and gene expression profiling of the 17q23 amplicon in human breast cancer. *Proc. Natl. Acad. Sci. USA*, **98**, 5711–5716 (2001).
31) Wu, G., Sinclair, C., Hinson, S., Ingle, J. N., Roche, P. C. and Couch, F. J. Structural analysis of the 17q22-23 amplicon identifies several independent targets of amplification in breast cancer cell lines and tumors. *Cancer Res.*, **61**, 4951–4955 (2001).
32) Racz, A., Brass, N., Heckel, D., Pahl, S., Remberger, K. and Meese, E. Expression analysis of genes at 3q26-q27 involved in frequent amplification in squamous cell lung carcinoma. *Eur. J. Cancer*, **35**, 641–646 (1999).
33) Sattler, H. P., Lensch, R., Rohde, V., Zimmer, E., Mese, E., Bonkhoff, H., Retz, M., Zwergel, T., Bex, A., Stoeckle, M. and Wullich, B. Novel amplification unit at chromosome 3q25-q27 in human prostate cancer. *Prostate*, **45**, 207–215 (2000).
34) Redon, R., Muller, D., Caulee, K., Wanherdrick, K., Abecassis, J. and du Manoir, S. A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains. *Cancer Res.*, **61**, 4122–4129 (2001).
35) Platzer, P., Upender, M. B., Wilson, K., Willis, J., Lutterbaugh, J., Nosrati, A., Willson, J. K. V., Mack, D., Ried, T. and Markowitz, S. Silence of chromosomal amplifications in colon cancer. *Cancer Res.*, **62**, 1134–1138 (2002).
36) Fujii, T., Dracheva, T., Player, A., Chacko, S., Clifford, R., Strausberg, R. L., Buetow, K., Azumi, N., Travis, W. D. and Jen, J. A preliminary transcriptome map of non-small cell lung cancer. *Cancer Res.*, **62**, 3340–3346 (2002).
37) Sato, K., Qian, J., Slezak, J. M., Lieber, M. M., Bostwick, D. G., Bergstralh, E. J. and Jenkins, R. B. Clinical significance of alterations of chromosome 8 in high-grade, advanced, nonmetastatic prostate carcinoma. *J. Natl. Cancer. Inst.*, **91**, 1574–1580 (1999).
38) Hidaka, S., Yasutake, T., Takeshita, H., Kondo, M., Tsuji, T., Nanashima, A., Sawai, T., Yamaguchi, H., Nakagoe, T., Ayabe, H. and Tagawa, Y. Differences in 20q13.2 copy number between colorectal cancers with and without liver metastasis. *Clin. Cancer Res.*, **6**, 2712–2717 (2000).
39) Nonet, G. H., Stampfer, M. R., Chin, K., Gray, J. W., Collins, C. C. and Yaswen, P. The ZNF217 gene amplified in breast cancers promotes immortalization of human mammary epithelial cells. *Cancer Res.*, **61**, 1250–1254 (2001).
40) Melhuish, T. A., Gallo, C. M. and Wotton, D. TGIF2 interacts with histone deacetylase 1 and represses transcription.

J. Biol. Chem., **276**, 32109–32114 (2001).
41) Wiener, J. R., Kerns, B. J., Harvey, E. L., Conaway, M. R., Iglehart, J. D., Berchuck, A. and Bast, R. C., Jr. Overexpression of the protein tyrosine phosphatase PTP1B in human breast cancer: association with p185c-erbB-2 protein expression. *J. Natl. Cancer Inst.*, **86**, 372–378 (1994).
42) Wiener, J. R., Hurteau, J. A., Kerns, B. J., Whitaker, R. S., Conaway, M. R., Berchuck, A. and Bast, R. C., Jr. Overexpression of the tyrosine phosphatase PTP1B is associated with human ovarian carcinomas. *Am. J. Obstet. Gynecol.*, **170**, 1177–1183 (1994).
43) Sakakura, C., Hagiwara, A., Yasuoka, R., Fujita, Y., Nakanishi, M., Masuda, K., Kimura, A., Nakamura, Y., Inazawa, J., Abe, T. and Yamagishi, H. Amplification and over-expression of the AIB1 nuclear receptor co-activator gene in primary gastric cancers. *Int. J. Cancer*, **89**, 217–223 (2000).
44) Zhou, H., Kuang, J., Zhong, L., Kuo, W. L., Gray, J. W., Sahin, A., Brinkley, B. R. and Sen, S. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat. Genet.*, **20**, 189–193 (1998).
45) Sakakura, C., Hagiwara, A., Yasuoka, R., Fujita, Y., Nakanishi, M., Masuda, K., Shimomura, K., Nakamura, Y., Inazawa, J., Abe, T. and Yamagishi, H. Tumour-amplified kinase BTAK is amplified and overexpressed in gastric cancers with possible involvement in aneuploid formation. *Br. J. Cancer*, **84**, 824–831 (2001).