2）・表　胃がん定型手術（D2）後のStage別（stageについては胃癌取り扱い規約第12版による）5年生存率（%）

Stage	5年生存率（%）	症例数
ⅠA	93.4	2030
ⅠB	87.0	725
Ⅱ	68.3	541
ⅢA	50.1	485
ⅢB	30.8	273
Ⅳ	16.6	440
Total	73.7	4494
日本胃癌学会全国登録1991年度症例：初発胃がん切除例、D2施行症例全例、消息不明率8.0%、手術死他病死含む。		

（日本胃癌学会編：胃がん治療ガイドライン　医師用，第2版，金原出版，2004，p.30.より）

・表　食道がん切除例病理学的Stage別5年生存率（%）

Stage	5年生存率（%）	症例数
0	72.3	232
Ⅰ	63.1	1163
ⅡA	49.2	1923
ⅡB	32.1	1614
Ⅲ	18.2	2381
Ⅳ	12.1	1003
Total	35.5	9143
日本食道疾患研究会全国登録1988～1994年度症例：食道がん切除例		

（The Japanese Society for Esophageal Disease：Comprehensive Registry of Esophageal Cancer in Japan（1988～1994），1st ed. The Jpn Soc for Espophag Dis，2000，p.31，p.32.より改変）

2）・The Japanese Society for Esophageal Disease：Comprehensive Registry of Esophageal Cancer in Japan（1998，1999），3rd ed. The Jpn Soc for Esophag Dis，2002.

・日本胃癌学会編：胃がん治療ガイドライン　医師用，第2版，金原出版，2004.　￥945（900）

・国立がんセンター内科レジデント編：がん診療レジデントマニュアル，第3版，医学書院，2003.　￥3,990（3,800）

・予後（5年生存率）は対象・合併療法などにより偏りがあることに注目するため、がん登録に関するサイトを参考に記す。

地域がん診療拠点病院院内がん登録 支援のページ

http://jcdb.ncc.go.jp/

地域がん診療拠点病院に関する厚生労働省からの通知等
http://jcdb.ncc.go.jp/tsuuti.htm
厚生労働省（ホームページ検索：がん登録）
http://www.mhlw.go.jp/

3）・一般的合併症を各臓器別に挙げる：脳血管・神経、心臓・血管、呼吸器、消化管、肝機能、腎機能、深在静脈血栓症・急性肺梗塞、内分泌。
・ジェラード・M・ドハティー著，安立健介訳：ワシントン外科マニュアル，7．周術期の医学的評価と管理，第3版，メディカル・サイエンス・インターナショナル，1998，p101-165．￥9,345（8,900）
同書は、教科書的マニュアルである The Washington Manual of Surgeryの翻訳本である。
・深在静脈血栓症・急性肺梗塞についてはガイドラインがあるので参考にする。
肺血栓塞栓症／深部静脈血栓症（静脈血栓塞栓症）予防ガイドラインのホームページ（ダイジェスト版）
http://www.jasper.gr.jp/daigest/
・術後長期障害：
肺がん；呼吸困難、呼吸訓練
胃がん；小（無）胃症状、ダンピング症候群
大腸がん；排便・排尿・性機能障害、人工肛門
など、代表的なものは国立がんセンターのホームページ（一般向けがん情報／看護・支持療法）を参照する。
・国立がんセンターのホームページ／一般向けがん情報／看護・支持療法
http://www.ncc.go.jp/jp/ncc-cis/pub/index/care.html

4）・手術により全身状態の改善が望める場合に適応がある。ただし、狭窄に対するステント留置術、栄養摂取補助の胃瘻造設術など、近年、IVR（Interventional Radiology）の技術の発達により行えることが増えてきた。それら治療法の選択を確実に行えるようにする（IVRの項Ⅶを参照）。

5）・消化管狭窄・出血に対する腫瘍切除（胃・大腸がんなど）
・消化管狭窄に対するバイパス手術（膵がん、結腸がんなど）
・人工肛門造設術（大腸がん・腹膜播種など）

6）・化学療法が主体の卵巣腫瘍における巨大腫瘍切除により根治性、脳腫瘍の一部ではQOL向上が期待されるが、議論の余地がある。
・国立がんセンター内科レジデント編：がん診療レジデントマニュアル，10．婦人科がん　卵巣がん，第3版，医学書院，2003，p116．￥3,990（3,800）
・BRAIN & NEUROSURGERY INFORMATION CENTERのホームページ／A, B, C's OF BRAIN TUMORS
http://www.brain-surgery.com/primer.html

7）・局所切除：乳房温存（乳がんなど）
・切除範囲縮小：肛門・骨盤神経機能温存術式（直腸がんなど）
・術前化学療法（＋放射線）により切除範囲縮小：喉頭温存（頸部食道がん）、関節温存（骨肉腫など）
・リンパ節郭清範囲縮小：センチネルリンパ節生検（乳がんなど）
・手術侵襲軽減：鏡視下手術による手術創縮小、術後早期回復
・再建手術：腹直筋皮弁による形成外科的舌再建（舌がん）、人工膀胱造設術（膀胱がんなど）

ELSEVIER

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Effects of degenerate oligonucleotide-primed polymerase chain reaction amplification and labeling methods on the sensitivity and specificity of metaphase- and array-based comparative genomic hybridization

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Abstract Degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) is often applied to small amounts of DNA from microdissected tissues in the analyses of chromosomal copy number with comparative genomic hybridization (CGH). The sensitivity and specificity in CGH analyses largely depend on the unbiased amplification and labeling of probe DNA, and the sensitivity and specificity should be high enough to detect one-copy changes in aneuploid cancer cells when accurate assessment of chromosomal instability is needed. The present study was designed to assess the effects of DOP-PCR and labeling method on the sensitivity of metaphase- and array-based CGHs in the detection of one-copy changes in near-tetraploid Kato-III cells. By focusing on several chromosomes whose absolute copy numbers were determined by FISH, we first compared the green-to-red ratio profiles of metaphase- and array-based CGH to the absolute copy numbers using the DNA diluted with varying proportions of lymphocyte DNA, with and without prior DOP-PCR amplification, and found that the amplification process scarcely affected the sensitivity but gave slightly lower specificity. Second, we compared random priming (RP) labeling with nick translation (NT) labeling and found that the RP labeling gave fewer false-positive gains and fewer false-negative losses in the detection of one-copy changes. In array CGH, locus-by-locus concordance between the DNAs with and without DOP-PCR amplification was high (nearly 100%) in the gain of three copies or more and the loss of two copies or more. This suggests that we could pinpoint the candidate genes within large-shift losses–gains that are detected with array CGH in microdissected tissues.

1. Introduction

Comparative genomic hybridization (CGH) is a powerful molecular cytogenetic method, enabling us to make genome-wide screening for copy-number losses and gains of chromosomal parts by single hybridization [1,2]. Recently, array CGH has evolved from CGH, using genomic DNA microarrays instead of metaphase spreads as the target of hybridization; this approach makes it possible to pinpoint the DNA clones that include the candidate genes [3–9]. Array as well as conventional CGH can be applied to genomic DNA extracted from both fresh [1] and paraffin-embedded tissues [10,11]. Applications of CGH in cancer genetics include not only inquiry into novel genes that are involved in hot spots of unbalanced genomic rearrangements [12] but also disclosure of tumor heterogeneity: regional heterogeneity of chromosomal constitution for the lineage analysis of individual cancers [13,14] and intertumoral heterogeneity for subclassification and prognostication of cancer [14–17]. In such studies, the degree of heterogeneity achieved depends on the detection sensitivity of loss–gain by CGH, which is affected by various materials factors (including factors such as the proportion of contaminated normal DNA or ploidy of tumor cell) and methodological factors, such as labeling methods.

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Recently, microdissection [18,19] is frequently used in CGH analyses on intratumoral heterogeneity [8,13,14, 20–23]. Microdissection is an important method for the purification of tumor cell types, through which we can link histological information to genomic DNA alterations. The DNA extracted from microdissected tissues, however, is too small in amount for standard CGH. To amplify small amounts of DNA efficiently, degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) is currently used [8,20,22,24–29]. But, it has not been fully examined to what extent this technique inevitably gives false-positive and false-negative results in CGH analysis.

Previous studies have examined this kind of problem in terms of all-or-none detection, although the detection sensitivity of loss–gain fundamentally depends on the ploidy of cells and how many copies of chromosomes or chromosomal regions the cells have lost or gained. In DNA-diploid tumors, one-copy loss or gain (with the green-to-red ratio G/R = 1/2 or 3/2, respectively) shows large-shift G/R ratios so long as the change is present in most of the cells in the sample tissue. In DNA-aneuploid tumors, both small-shift changes (such as 1/3 and 1/4 in G/R ratio) and large-shift changes (such as 2/3 and 2/4 in G/R ratio) may be detected [30–32]. This means that studies with different detection sensitivity may yield different results, which may be one of the factors underlying the inconsistency of CGH data. For the present study, therefore, we examined the copy-number-dependent detection sensitivity in metaphase and array CGHs, using a near-tetraploid cell line, KATO-III. In near-tetraploid cells, we can readily distinguish between one-copy changes and two-copy changes of chromosomal DNA, of which the approximate G/R ratios are $1 \pm (1/4)$ and $1 \pm (2/4)$, respectively. We confined the examination to a number of chromosomes, whose absolute copy numbers were determined by FISH with painting and telomeric probes on metaphase spread, and compared the G/R ratio profile of the tumor DNA with the values expected from the absolute copy number of these chromosomes.

The major technical factors that affect the detection sensitivity and specificity of CGH may reside in the process of probe labeling. We compared two labeling methods in the present study: nick translation and random priming. The process of DOP-PCR amplification, which may possibly modify the DNA constitution, might also affect the specificity of CGH. To examine this possibility, we set up a model study to assess whether biased amplification of certain components of tumor DNA occurs; for this study, DNA of KATO-III was admixed with varying proportions of normal lymphocyte DNA before DOP-PCR amplification [33]. We also compared the CGH results between the PCR products of the exponential phase and plateau phase, using a real-time PCR method. Based on these examinations, we tried to clarify the effects of DOP-PCR amplification and labeling on the sensitivity and specificity of metaphase- and array-based CGH, and propose a DOP-PCR CGH protocol modified for detection of one-copy changes in DNA-aneuploid tumors.

2. Materials and methods

2.1. Cell line

We used a gastric cancer cell line, KATO-III, which was maintained in Dulbecco's Modified Eagle Medium with L-Glutamine and HEPES, supplemented with 10% heat-inactivated fetal calf serum. Metaphase spreads were prepared by a standard method with Colcemid. This cell line is DNA-near tetraploid [30].

2.2. Determination of chromosomal copy number by dual-color FISH with centromeric and painting or telomeric probes

The smear slides of the cell line were covered by 5 μL dual hybridization mixture containing a pair of painting and centromeric probes, which were labeled with different fluorochromes. The painting probes of chromosomes 8 and 18 were labeled with SpectrumOrange; for chromosomes 2, 3, 4, 6, 7, 9, 11, and 13, the probes were labeled with SpectrumGreen (Vysis, Downers Grove, IL). For the structural analyses of chromosomes 3 and 11, we used telomeric probes of short and long arms labeled with SpectrumOrange and SpectrumGreen (Vysis), respectively. The slides and probes were denatured at 74 °C for 30–120 seconds and hybridized for 1–2 days. Posthybridization washes were performed according to the manufacturer's protocol. The copy numbers of chromosome segments were determined by comparing FISH results with CGH profiles.

2.3. DNA extraction and sample preparation

DNA was extracted by the phenol–chloroform method after digestion with 200 μg/mL proteinase K at 42 °C for 24 hours. After the DNA concentration was determined by means of a spectrophotometer (Ultraspec III; Pharmacia, England), each DNA sample was diluted to 0.2 μg/μL with Tris–EDTA buffer (pH 7.5). Taking the ploidy into consideration, we made the samples with varying percentages of Kato-III cells by adding normal DNA extracted from peripheral blood lymphocytes of a healthy male (Table 1).

Table 1
Composition of the samples tested by CGH and array CGH

Tumor nuclei, %	DNA, NL:Kato-III
50	1:2
60	1:3
80	1:8
100	0:1

Normal lymphocyte (NL) is DNA-diploid; KATO-III is DNA-tetraploid.

2.4. DOP-PCR amplification of DNA

DNA amplification by DOP-PCR was separated into two steps. The reagents, volumes, and reaction conditions are given in Table 2. PCR was performed on a thermocycler (Model PTC100, MJ Research, Watertown, MA) and LightCycler (Roche, Mannheim, Germany). Initial 5 cycles were in a reaction mixture using TaKaRa ExTaq (Takara Bio, Otsu, Japan) in a low-stringency (degenerate) condition on the MJ thermocycler (step 1). The following cycles were in a 25-μL or 50-μL reaction volume in a high-stringency condition either on the LightCycler or on the MJ thermocycler, respectively (step 2). To analyze amplification bias, we monitored the amount of PCR product by LightCycler as the fluorescence of SYBR Green in one of the tubes, and took a PCR product in the exponential phase just before the transition to the plateau phase and another product in the plateau phase.

2.5. Probe DNA labeling

By nick translation reaction (NT), DOP-PCR amplified tumor and normal DNAs were labeled with fluorescein isothiocyanate (FITC)-12-dUTP and tetramethyl rhodamine isothiocyanate (TRITC)-5-dUTP (Roche), respectively, as described previously [30]. FITC-dUTP and TRITC-dUTP were substituted with Cy3-dCTP and Cy5-dCTP, respectively (PerkinElmer Life Sciences, Boston, MA), in an alternative protocol. Random priming (RP) labeling was performed according to the manufacturer's protocol (Vysis). Briefly, 100 ng each of nonamplified or DOP-PCR-amplified tumor DNA and normal reference DNA of the same sex (male) were labeled with Cy3- and Cy5-dCTPs (PerkinElmer), respectively, by random priming reaction (Vysis Random Priming Labeling Kit) followed by DNase digestion.

Table 2
DOP-PCR amplification of probe DNA

	First PCR		Second PCR	
	MJ cycler	MJ cycler	MJ cycler	LightCycler
Template DNA	2 ng	2 ng	10 μL FPP	5 μL FPP
TaKaRa ExTaq (5 units/μL)	0.5 μL	0.5 μL	1 μL	0.5 μL
10× ExTaq Buffer (Mg^{2+} free)	1.0 μL	0.5 μL	4 μL	2 μL
25 mmol/L $MgCl_2$	1.2 μL	0.6 μL	6 μL	3 μL
2.5 mmol/L dNTP mixture	1.0 μL	0.5 μL	4 μL	2 μL
50 mmol/L universal primer	0.3 μL	0.2 μL	1 μL	0.6 μL
SYBR Green	—	—	—	1 μL[a]
Double-distilled water	To volume	To volume	To volume	To volume
Total volume	10 μL	5 μL	50 μL	25 μL

First PCR: Initial step, 94 °C for 10 min; then five cycles of 94 °C for 1 min, 25 °C for 1 min, 3 min ramp from 25 °C to 74 °C, and 74 °C for 2 min; finally, 4 °C stock. Second PCR: Initial step, 94 °C 5 min; then 35 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 2 min; finally 72 °C for 10 min; then, 4 °C stock.

Abbreviations: FPP, first PCR product.

[a] SYBR Green was added only in the monitor tube.

2.6. Metaphase CGH

The labeled tumor and reference DNAs (1–2 μg each of NT-labeled DNA or 200 ng each of RP-labeled DNA) admixed with 10 μg human Cot-1 DNA (Life Technologies, Gaithersburg, MD) were ethanol-precipitated and dissolved in 50% formamide–10% dextran sulfate. The processes of denaturation, hybridization, and image analysis were as described previously [20]. Images were captured with a fluorescence microscope (Nikon FX; Nikon, Yokohama, Japan) equipped with a charge-coupled device camera (Sensys SS1401-E; Photometrics, Munich, Germany) and filter systems for cy3–cy5 as well as for green–red fluorescence. Gains and losses in DNA sequence copy number were defined by G/R of >1.2 and <0.8 (0.85), respectively. High-level gains (amplifications) were defined by G/R $\geqslant$ 1.5.

2.7. Array CGH

After denaturation at 80 °C for 10 minutes, 100 ng RP-labeled DNAs or 1 μg NT-labeled DNAs were preincubated in Microarray hybridization buffer (Vysis) containing Cot-1 DNA at 37 °C for 2 hours. The processes of hybridization and image analysis were as described previously [13]. We used GenoSensor Array 300 (Vysis) spotted with 287 target DNA clones in triplicate. (The clone list is available on the Internet at http://www.abbottmoleculardiagnostics.com/PDF/GenoSensor300ClonesandKey-July2004.pdf.)

We compared two threshold criteria: (a) T/R (tumor–reference ratio) > 1.2 and T/R < 0.8 (0.85), corresponding to gain and loss, respectively, and (b) $P < 0.01$, where the P value of each set of target spots was automatically assigned by the GenoSensor system. With both criteria, we excluded from the analysis the positive loci in normal-versus-normal experiments (N–N), except in loci that showed gain in spite of loss in N–N or vice versa. The sensitivity in detection of a certain copy number change was assessed as the ratio of the number of positive loci to the number of informative loci within the areas in which metaphase CGH showed the same copy number change.

Between the array CGH with DOP-PCR amplification and that without, we compared the positive loci throughout the total autosomal chromosomes locus by locus and calculated the rate of concordance between them.

3. Results

3.1. Determination of the copy number of chromosomal parts by FISH and CGH

We compared the CGH profile and the number and size of FISH signals with painting and centromeric probes to determine the copy number of chromosomal parts in

chromosomes 2, 3, 4, 6, 7, 8, 9, 11, 13, and 18 (Fig. 1). For chromosomes 3 and 11, the rearrangements were so complicated that it was necessary to determine telomeric copy numbers of short and long arms. It was found that Kato-III showed trisomy 2 with an additional one-copy of whole 2p, another copy of 2p telomeric tip, and an interstitial deletion

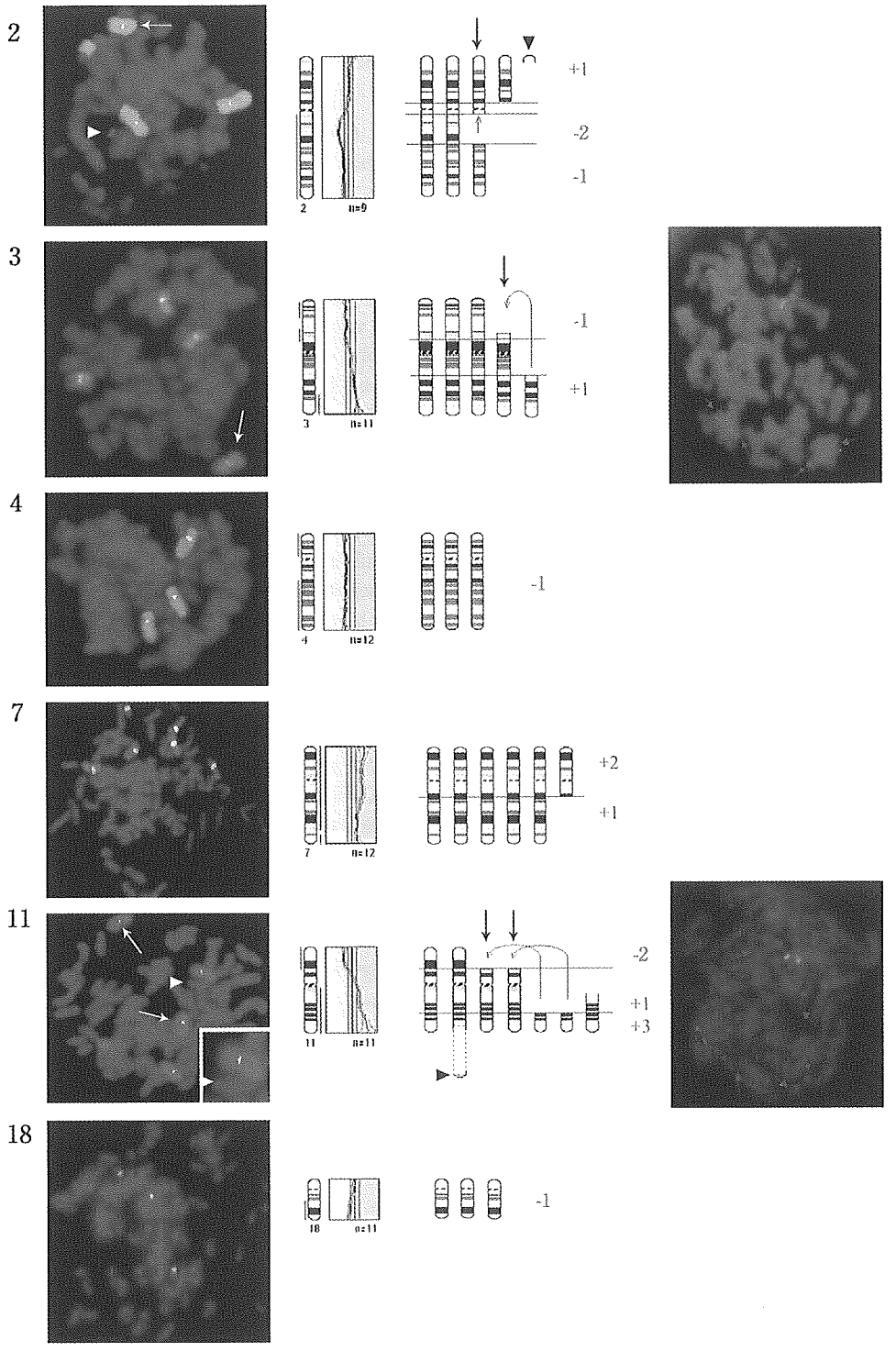


Fig. 1. Absolute copy numbers of chromosomal parts in chromosomes 2, 3, 4, 7, 11, and 18 determined by comparing painting and telomeric FISH signal patterns to CGH profiles with random primer labeling and the thresholds of G/R > 1.2 and G/R < 0.85. Green and red telomeric signals (indicated by arrows of the same color) correspond to the telomeres of the short and long arms, respectively. The copy numbers of chromosomal parts determined are shown as ideograms. One of the four chromosomes 3 and two of the four chromosomes 11 have two red telomeres, reflecting the replacement of the short arms by extra copies of the telomeric parts of the long arm.

in one-copy of the proximal 2q, tetrasomy 3 with a Robertsonian translocation with the short arm (p14~pter) replaced by the long arm (q22~qter), trisomy 4, hexasomy 7 with one-copy loss of the whole 7q, and trisomy 18. Chromosome 11 showed tetrasomy with an additional distal 11q. In two of these chromosomes, 11q22~qter replaced 11p14~pter. Chromosomes 6, 8, 9, and 13 showed tetrasomy without structural abnormalities.

3.2. Examination of amplification bias

The product of our DOP-PCR amplification was 300 to several thousand base pairs long, enough for nick labeling. The DOP-PCR amplification scarcely affected on the G/R ratio profiles of CGH (Fig. 2A; Table 3). When the template concentration ranged from 2 to 20 ng, the SYBR Green fluorescence reached a plateau at the 15th to the 20th cycle. Thereafter, the fluorescence intensity gradually reduced to the half at the 35th cycle (probably reflecting an increase of single-stranded DNA), though the total DNA amount remained constant from the 15th to 35th cycle. Virtually no difference was observed between the CGH profile using DOP-PCR products in the exponential phase (10th–15th cycle) and that using the products in the plateau phase (20th–35th cycle) (data not shown). The cycle number of the second (high-stringency) phase of DOP-PCR was fixed to 35 cycles in the experiment summarized in Table 3.

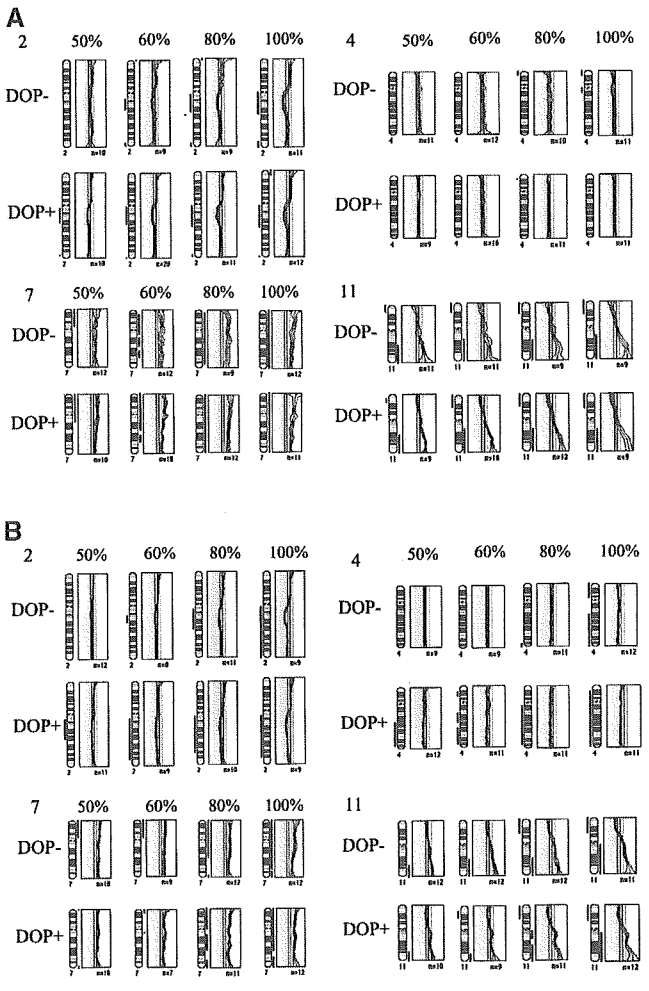


Fig. 2. CGH profiles using (A) nick translation-labeled or (B) random priming-labeled DNA with and without DOP-PCR amplification. Significant gain and loss were assessed by G/R > 1.2 and G/R < 0.85 thresholds, respectively. The vertical bars on the left and right sides of each chromosome ideogram indicate the regions of losses and gains, respectively.

3.3. Effects of DOP-PCR amplification and labeling method on the sensitivity of metaphase CGH

The degree of reduction in the shift distance of the G/R ratio by adding normal DNA was not affected by the use of DOP-PCR-amplified tumor DNA (Table 3).

In RP labeling, the actual G/R ratio in copy-number gain was very close to the one calculated from the absolute copy number and the percentage of cancer cells (Fig. 2A; Table 3). In NT labeling, on the other hand, the detection sensitivity of one-copy gain was higher than expected (Table 3); one-copy gain was partially detected even in the condition of 60% cancer cells, of which the calculated G/R ratio was 1.15. In copy-number loss, the shift distance of actual G/R ratio in both NT labeling and RP labeling was smaller than calculated; however, the detection sensitivity of loss was higher in RP labeling (Fig. 2B). In RP labeling but not in NT labeling, one-copy losses became significant mostly when the threshold for loss was shifted to 0.85 in 100% tumor samples—and partly significant in the 80% tumor samples (Table 3). The control samples (N–N) with RP labeling did not show any loss or gain even when the threshold for the loss was set at 0.85; with NT labeling, however, the G/R ratios in N–N were sometimes less than 0.85 (data not shown).

3.4. Effects of DOP-PCR amplification and labeling method on the specificity of metaphase CGH

False-positive changes (mostly equivalent to one-copy gains) in the chromosomes without loss–gain were more common in NT labeling than in RP labeling (Table 3). These changes were different from nonspecific ones which remained unchanged irrespective of the proportion of normal DNA contamination. NT labeling showed nonspecific losses at 1p36, 16p, and chromosomes 19 and 22, as pointed out previously [33]; RP labeling showed nonspecific gains at the same places. There was additional nonspecific gain at 9q telomeric tip in RP labeling (Table 3).

3.5. Effects of labeling method on the sensitivity and specificity of array CGH

When the threshold was $P < 0.01$, the array CGH hardly detected one-copy changes with RP or NT labeling, and false-positive signals were scarce (Fig. 3A, B; Table 4). When the threshold was T/R >1.2 or <0.85, the detection

Table 3
Effects of DNA amplification by DOP-PCR and percentage of cancer cells on the sensitivity of metaphase and array CGH to detect varying copy numbers of gains and losses, which were determined by FISH

		DOP−						DOP+						Expected results (G/R ratio)			
Nick translation labeling		CGH (% cancer cells)				array CGH		CGH (% cancer cells)				array CGH		CGH (% cancer cells)			
Results of FISH		50	60	80	100	$P<0.01$	T/R	50	60	80	100	$P<0.01$	T/R	50	60	80	100
Loss of one copy	2q (distal)	–/–*	–/–	–/TR−	–/–	P−	TR−/TR+	–/–	–/–	–/–	–/–	—	–/–	–/–	–/TR−	TR−/TR+	TR+/TR+
	3p (distal)	–/–	–/–	–/–	–/TR−	P−	TR+/TR−#	–/–	–/–	–/–	–/–	P−	TR−/TR−	(0.875)	(0.85)	(0.8)	(0.75)
	4	–/–	–/–	–/–	–/TR−	—	TR−/TR−#	–/–	–/–	–/–	–/–	—	–/TR−				
	18	–/–	–/–	–/–	–/TR−	—	TR−/TR+	–/–	–/–	–/–	–/–	—	TR−/TR−				
Loss of two copies	2q (proximal)	–/–	–/TR+	TR+/TR+	TR+/TR+	P+	TR+/TR+	–/TR+	TR+/TR+	TR+/TR+	TR+/TR+	P−	TR+/TR+	TR+/TR+	TR+/TR+	TR+/TR+	TR+/TR+
	11p (distal)	–/–	TR−/TR+	TR+/TR+	TR+/TR+	—	TR+/TR+#	–/TR−	TR+/TR+	TR+/TR+	TR+/TR+	P−	TR−/TR+	(0.75)	(0.7)	(0.6)	(0.5)
Gain of one copy	3q (distal)	—	TR−	TR+	TR+	P+	TR+	TR−	TR+	TR+	TR+	—	TR+	—	—	TR−	TR+
	7q (distal)	—	TR−	TR+	TR+	P−	TR+	—	TR−	TR+	TR+	P−	TR+	(1.125)	(1.15)	(1.2)	(1.25)
	11q (proximal)	—	TR−	TR−	TR+	P−	TR+	—	TR−	TR−	TR+	P−	TR+				
Gain of two copies	7p/q (proximal)	TR+	TR+	TR+	TR+	P+	TR+	TR+	TR+	TR+	TR+	P−	TR+#	TR+ (1.25)	TR+ (1.3)	TR+ (1.4)	TR+ (1.5)
Gain of three copies	11q (distal)	TR+	TR+	TR+	TR+	P+	TR+	TR+	TR+	TR+	TR+	P+	TR+	TR+ (1.375)	TR+ (1.45)	TR+ (1.6)	TR+ (1.75)
No loss/gain	6	—	—	—	—	P−#	TR−#	—	—	TR−#	TR−#	—	TR−#	—	—	—	—
	8	—	—	—	—	P−#	TR−#	—	—	TR−#	TR−#	P−#	TR−#	(1.0)	(1.0)	(1.0)	(1.0)
	9	—	—	TR−#	TR−#	—	—	—	TR−#	TR−#	TR−#	P−#	TR−#				
	13q	—	—	—	TR+#	—	TR−#	—	—	TR−#	TR+#	—	—				
		DOP−						DOP+						Expected results (G/R ratio)			
Random priming labeling		CGH (% cancer cells)				array CGH		CGH (% cancer cells)				array CGH		CGH (% cancer cells)			
Result of FISH		50	60	80	100	$P<0.01$	T/R	50	60	80	100	$P<0.01$	T/R	50	60	80	100
Loss of one copy	2q (distal)	–/–	–/–	–/–	TR−/TR+	—	TR−/TR+	–/–	–/TR−	–/TR−	–/TR+	—	TR−/TR+	–/–	–/TR−	TR−/TR+	TR+/TR+
	3p (distal)	–/–	–/–	–/–	TR−/TR+	—	TR−/TR+	–/–	–/TR−	–/TR−	–/TR+	—	TR+/TR+	(0.875)	(0.85)	(0.8)	(0.75)
	4	–/–	–/–	–/TR−	TR−/TR+	—	TR−/TR−	–/TR−	–/TR+	–/TR+	TR+/TR+	—	TR−/TR+				
	18	–/–	–/–	–/TR−	TR−/TR+	—	TR−/TR+	–/–	–/TR−	–/TR+	–/TR+	—	TR−/TR+				
Loss of two copies	2q (proximal)	–/–	–/TR+	TR+/TR+	TR+/TR+	P−	TR−/TR+	–/TR+	TR+/TR+	TR+/TR+	TR+/TR+	P+	TR+/TR+	TR+/TR+	TR+/TR+	TR+/TR+	TR+/TR+
	11p (distal)	–/–	–/–	TR+/TR+	TR+/TR+	P+	TR+/TR+	–/–	–/TR+	TR+/TR+	TR+/TR+	—	TR+/TR+	(0.75)	(0.7)	(0.6)	(0.5)
Gain of one copy	3q (distal)	—	—	TR−	TR+	—	—	—	—	TR−	TR−	P+	TR+	—	—	TR−	TR+
	7q (distal)	—	—	TR−	TR+	—	TR+	—	—	TR+	TR+	—	TR−	(1.125)	(1.15)	(1.2)	(1.25)
	11q (proximal)	—	—	—	TR+	P−	TR+	—	—	TR+	TR+	P−	TR+				

(*Continued*)

Table 3
Continued

		DOP−					DOP+					Expected results (G/R ratio)			
Random priming labeling		CGH (% cancer cells)				array CGH	CGH (% cancer cells)				array CGH	CGH (% cancer cells)			
Result of FISH		50	60	80	100	P<0.01 T/R	50	60	80	100	P<0.01 T/R	50	60	80	100
Gain of two copies	7p/q (proximal)	TR+	TR+	TR+	TR+	P− TR+	TR−	TR−	TR+	TR+	P− TR+	TR+ (1.25)	TR+ (1.3)	TR+ (1.4)	TR+ (1.5)
Gain of three copies	11q (distal)	TR+	TR+	TR+	TR+	P+ TR+	TR+	TR+	TR+	TR+	P+ TR+	TR+ (1.375)	TR+ (1.45)	TR+ (1.6)	TR+ (1.75)
No loss/gain	6	—	—	—	—	— TR−#	—	—	—	—	— TR−#	—	—	—	—
	8	—	—	—	TR−#	— —	—	TR−#	TR−#	—	P−# TR−#	(1.0)	(1.0)	(1.0)	(1.0)
	9	—	—	—	—	— TR−#	TR−¶	TR−¶	TR−¶	TR−¶	— TR−#				
	13q	—	—	—	—	— —	—	—	—	—	— —				

TR+, 50% or more loci (proportion) of individual chromosomal part showed significant shift to gain or loss evaluated by T/R>1.2 or T/R<0.8, respectively.
TR−, Less than 50% of loci (proportion) of individual chromosomal part showed significant shift to gain–loss.
P+, 50% or more loci showed significant shift to gain–loss evaluated by P<0.01.
P−, Less than 50% of the loci showed significant shift to gain–loss evaluated by P<0.01.
—, Not significant.
*, The thresholds for loss are 0.8 and 0.85 on the left and right sides of slash, respectively. #, False positive. ¶, Nonspecific change.

sensitivity became higher (Fig. 3C, D), and the number of informative loci were comparable to that in $P < 0.01$ (Table 4). In RP labeling, the detection sensitivity was quite similar between the array CGH with and that without DOP-PCR; however, false-positive changes (mostly gains) were increased, except in RP labeling without DOP-PCR amplification (Fig. 3D; Table 4). Using NT, the sensitivity for detecting losses was reduced, particularly in DOP-PCR-amplified DNA, but false-positive spots were detected irrespective of DOP-PCR, not only in the regions without loss–gain at the frequency of 29%, but also in the midst of the loss–gain regions, as the opposite-color gain–loss signals (Fig. 3C; Table 4). The sensitivity of array CGH was symmetric in loss and gain, unlike that in metaphase CGH.

To simulate the DNA extracted from primary tumor samples, we prepared DNA at the purity of 80% cancer cells, labeled by RP. With this DNA, the detection sensitivity was reduced, and false-positive gains were occasionally seen in chromosomes 6, 8, 9, and 13 (Fig. 3E). In the regions of loss–gain of the absolute copy number, there were also a few opposite color gain–loss spots.

3.6. *Effect of DOP-PCR on the locus-by-locus consistency in array CGH*

When the threshold was $P < 0.01$, 15/34 (44%) of the positive loci that showed significant losses–gains in the array CGH with DOP-PCR coincided locus-by-locus to the positive loci in that without DOP-PCR (Table 5; Fig. 3B). The application of the threshold of T/R > 1.2 and T/R < 0.85 slightly improved the concordance of positive loci, 53/83 (64%), between the CGH arrays with and without DOP-PCR (Table 5; Fig. 3D). The concordance was lower in NT labeling (Table 5). In the loci examined by FISH, complete concordance between the array CGH with and that without DOP-PCR was in the regions of three-copy gain and two-copy loss. The concordance of two-copy gain and one-copy loss was 5/7 (71%) and 6/10 (60%), respectively. Few of the loci with one-copy gain was concordant (Fig. 3D).

4. Discussion

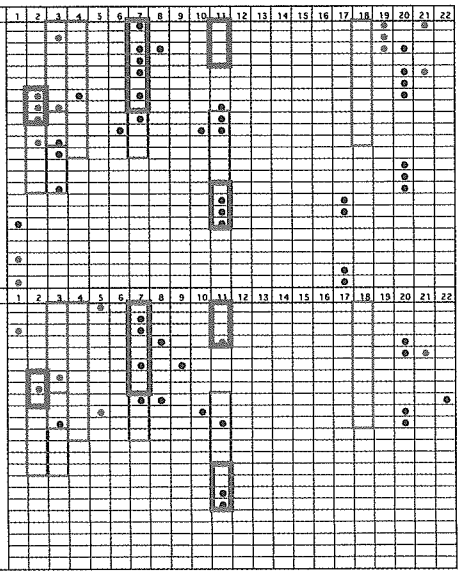
The present study was designed to simulate the CGH of primary tumors and to assess whether DOP-PCR amplification

Fig. 3. Array CGH results for different threshold criteria with and without DOP-PCR amplification: (A, B) for $P < 0.01$ or (C, D, E) for tumor/reference criteria of T/R > 1.2 for gain and T/R < 0.85 for loss. Each circle represents the CGH signal (red: copy-number loss; green: copy-number gain; yellow: no significant change) of the locus that corresponds to microarray spots. These loci are rearranged in each chromosome so that the uppermost and lowermost spots are the telomeric tips of the short arm and long arm, respectively. The positive loci in the N–N experiment were considered not informative and were excluded from the figure. The DNAs used were from 100% tumor cells in (A–D) and 80% tumor cells in (E).

A P<0.01

DOP-:
After subtraction of the loci positive in N(DOP-)/N(DOP-), nick translation labeling

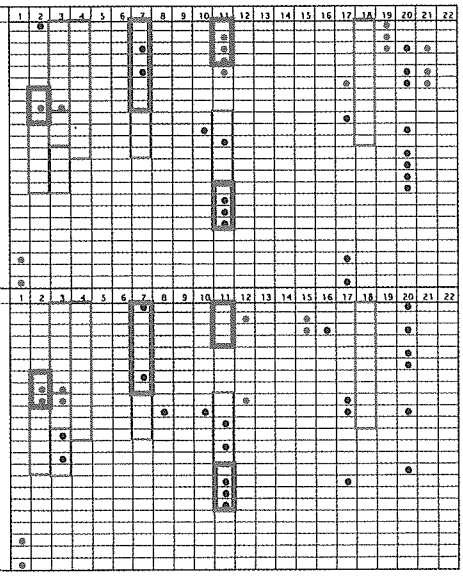
DOP+:
After subtraction of the loci positive in N(DOP+)/N(DOP+), nick translation labeling



B P<0.01

DOP-:
After subtraction of the loci positive in N(DOP-)/N(DOP-), random priming labeling

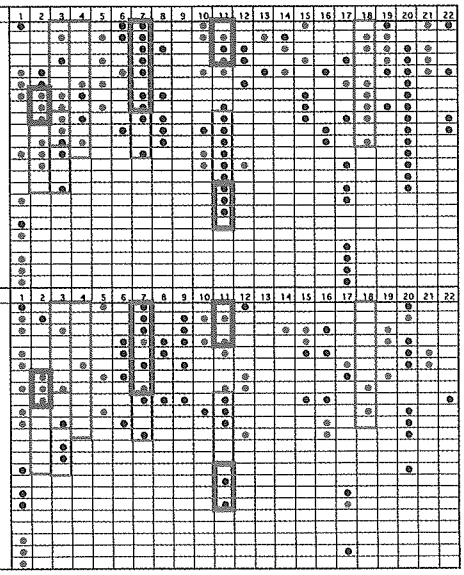
DOP+:
After subtraction of the loci positive in N(DOP+)/N(DOP+), random priming labeling



C T/R>1.2 T/R<0.85

DOP-:
After subtraction of the loci positive in N(DOP-)/N(DOP-), nick translation labeling

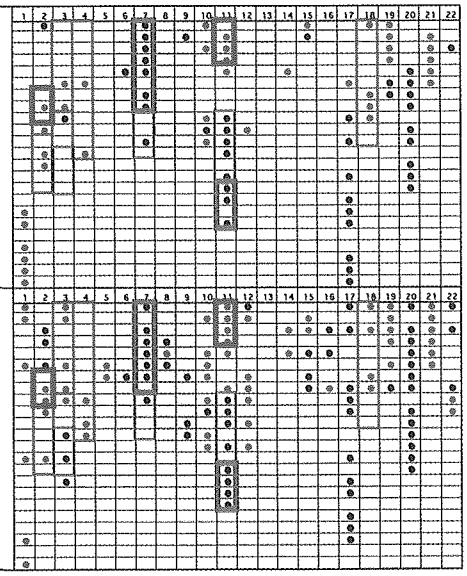
DOP+:
After subtraction of the loci positive in N(DOP+)/N(DOP+), nick translation labeling



D T/R>1.2 T/R<0.85

DOP-:
After subtraction of the loci positive in N(DOP-)/N(DOP-), random priming labeling

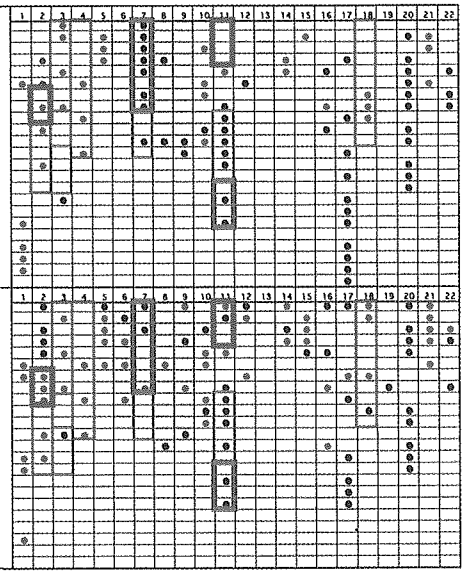
DOP+:
After subtraction of the loci positive in N(DOP+)/N(DOP+), random priming labeling



E T/R>1.2 T/R<0.85

DOP-:
After subtraction of the loci positive in N(DOP-)/N(DOP-), random priming labeling

DOP+:
After subtraction of the loci positive in N(DOP+)/N(DOP+), random priming labeling



and labeling of DNA of heterogeneous composition exert any influence on the detection sensitivity and specificity of CGH. We found that, in metaphase CGH, the DOP-PCR amplification of DNA scarcely affected the profile of the G/R ratio and the size of reduction in the G/R ratio by adding varying proportions of normal DNA. The G/R ratio profiles of CGH using DOP-PCR products did not show any difference between the products of the exponential phase and that of the plateau phase. These findings indicate that the amplification of DNA of heterogeneous composition was not biased, retaining the composition of copy-number changes even in the plateau phase of PCR. This may be because the DNA fragments generated in the first phase of DOP-PCR evenly represent the whole DNA sequence and because the exponential amplification of each DNA fragment in the second phase ceases in a completely synchronous manner (due to competitive inhibition of priming by PCR product). As will be discussed, however, some false-positive signals appeared after DOP-PCR amplification.

To assess the detection sensitivity and specificity of DOP-PCR CGH, we compared the G/R ratio profile to the absolute copy numbers of chromosomes that were determined by FISH. In NT labeling, the actual mean G/R ratio in the areas of one-copy gain exceeded 1.2 when the proportion of normal cells was 20% or less, as expected from the calculations that, in tetraploid tumor cells, the G/R ratio corresponding to one-copy gain is 5/4 (1.25) without concomitance of normal cells and becomes 1.2 in the presence of 20% contamination of normal cells. On the other hand, it was difficult for metaphase CGH with or without DOP-PCR amplification to detect one-copy loss of chromosomal part in DNA-tetraploid tumor cells. We thus tried an alternative CGH protocol, changing the labeling method.

As will be discussed, the sensitivity of array CGH with RP labeling to detect losses was comparable to that for detection of gains. It was expected from this fact that metaphase CGH after RP labeling or NT labeling with Cy3–Cy5 might have the sensitivity comparable to array CGH.

Table 4
Detection sensitivity of array CGH at chromosome level

	Informative locus number					
	$P < 0.01$			T/R > 1.2 or < 0.85		
Pattern of copy number change	Loss	Gain	Total loci	Loss	Gain	Total loci
Nick translation without DOP-PCR						
−1 (2q, 3p, 4, 18)	3 (8[a])	**1**	36	15 (43)	**3**	35
−2 (2q, 11p)	3 (50)	0	6	4 (67)	**1**	6
+1 (3q, 7q, 11q)	0	5 (36)	14	0	10 (71)	14
+2 (7p–q)	0	5 (63)	8	0	7 (88)	8
+3 (11q)	0	3 (75)	4	0	3 (100)	3
± 0 (6, 8, 9, 13q)	0	**2**	38	**2**	**9**	38
Total			106			104
Nick translation with DOP-PCR						
−1 (2q, 3p, 4, 18)	1 (3)	0	35	5 (17)	0	31
−2 (2q, 11p)	2 (29)	0	7	5 (83)	0	6
+1 (3q, 7q, 11q)	0	2 (15)	13	0	7 (54)	13
+2 (7p–q)	0	3 (38)	8	2	5 (63)	8
+3 (11q)	0	2 (67)	3	0	2 (100)	2
± 0 (6, 8, 9, 13q)	0	**3**	41	0	**12**	41
Total			107			101
Random priming without DOP-PCR						
−1 (2q, 3p, 4,18)	1 (3)	0	37	11 (35)	0	31
−2 (2q, 11p)	4 (67)	0	6	4 (67)	0	6
+1 (3q, 7q, 11q)	0	1 (8)	12	0	5 (50)	10
+2 (7p–q)	0	2 (25)	8	0	7 (88)	8
+3 (11q)	0	3 (75)	4	0	3 (100)	3
± 0 (6, 8, 9, 13q)	0	0	41	0	**2**	33
Total			108			91
Random priming with DOP-PCR						
−1 (2q, 3p, 4, 18)	1 (3)	0	31	13 (38)	0	34
−2 (2q, 11p)	2 (50)	0	4	6 (86)	0	7
+1 (3q, 7q, 11q)	0	4 (36)	11	0	7 (54)	13
+2 (7p–q)	0	2 (33)	6	0	6 (75)	8
+3 (11q)	0	3 (75)	4	0	4 (100)	4
± 0 (6, 8, 9, 13q)	0	**1**	37	**1**	**6**	33
Total			93			99

Gains and losses were defined by tumor–reference ratios (T/R) of >1.2 and <0.85, respectively, or by $P < 0.01$. Boldface type indicate false-positive results.
[a] Percentage in the total loci of the chromosomal regions with each pattern of copy number change.

Table 5
Locus-by-locus comparison of loss or gain between the array CGHs with and without DOP-PCR amplification

		$P < 0.01$			T/R > 1.2 or < 0.85		
Labeling method	DOP-PCR	Loss	Gain	Total	Loss	Gain	Total
Nick translation	−	13	31	44	57	79	136
	+	7	17	24	45	50	95
	Concordance	2 (15[a])	7 (23)	9 (20)	21 (37)	25 (32)	46 (34)
Random priming	−	15	19	34	40	43	83
	+	10	21	31	63	62	125
	Concordance	4 (27)	11 (58)	15 (44)	26 (65)	27 (63)	53 (64)

[a] Ratio (%) of the number of concordant loci to the positive loci without DOP-PCR.

Whereas Cy3–Cy5 labeling by NT gave no improvement (data not shown), Cy3–Cy5 labeling by RP labeling showed some improvements—although still insufficient. Using an alternative threshold for the loss, G/R < 0.85 (which had no effect on the N–N control results), we could detect one-copy loss so long as the purity of tumor cells exceeded 80%. On the other hand, in NT labeling, similar modification of the threshold caused false-positive losses in CGH of N–N control samples.

In NT labeling, the sensitivity for a detection of gain was higher than the expected one (Table 3), and may reflect a tendency of NT labeling to give a false-positive gains, which was demonstrated in the chromosomes without loss or gain (in particular, 13q). In RP labeling, on the other hand, the sensitivity of detection of gain almost conformed to the expected one. Additionally, the probe DNA needed was smaller in amount (due to higher labeling efficiency) and the standard deviation of the G/R ratio profile was smaller than in NT labeling. Thus, RP labeling may be preferable in metaphase as well as array CGH for detection of one-copy changes in DNA aneuploid tumors; however, with DOP-PCR amplification, false-positive gains were detected in chromosomes 8 and 9. In particular, false-positive gain at the 9q telomeric tip was irrespective of the proportion of concomitant normal DNA. This region should be excluded as a nonspecific change in addition to 1p, 16p, and chromosomes 19 and 22 [33].

Array CGH with RP-labeled unamplified or amplified probe DNA scarcely detected one-copy changes and only partially did two-copy changes in the condition of $P < 0.01$. This criterion may be too stringent, though it gave scarce false-positive gains without DOP-PCR. We thus used less stringent thresholds: T/R > 1.2 or T/R < 0.85, as in the metaphase CGH, provided the spots that were positive in N–N hybridization were excluded. This effectively removed the noise that inevitably accompanies the enhanced detection sensitivity. With these new thresholds, the array CGH could detect one-copy losses at the sensitivity of 35%, two-copy losses at 67%, one-copy gains at 50% and two-copy gains at 88% (Fig. 3D; Table 4). DOP-PCR-amplified DNA gave the sensitivity comparable to unamplified DNA but did slightly lower specificity.

At the individual locus level, however, the loci of one-copy losses–gains in the chromosomal parts examined were not consistent between the array with and without DOP-PCR amplification, and those of two-copy gains were only partially consistent between the two, whereas high-level gains (of three copies or more) at 10q26 and 11q and large-shift losses of two copies or more in near-tetraploid cells were easily consistent with or without DOP-PCR amplification (Fig. 3). Although array CGH is superior to metaphase CGH in its ability to detect amplicons that are too short to identify by metaphase CGH [7], and can pinpoint the smaller DNA segment that contains target genes, the signal-to-noise ratio appears to be inferior to metaphase CGH. This point needs further study with repeated array CGH experiments.

In summary, at the level of chromosomal region (Tables 3, 4), metaphase and array CGH with RP labeling and without DOP-PCR gave the best sensitivity and specificity. Even RP labeling, however, was not sufficient and required modification of thresholds for the detection of one-copy losses. As to the specificity, false-positive gains were associated with NT labeling and DOP-PCR amplification. The process of DOP-PCR did not give any biased amplification, but increased the noise level, which caused reduction in the locus-by-locus concordance between the result with and that without DOP-PCR amplification. This point was partially improved by the use of RP labeling, but has to be further improved so that array-based DOP-PCR CGH become a reliable tool for detection of one-copy changes in DNA-aneuploid cells, which is applicable to microdissected samples.

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ORIGINAL REPORT

Nationwide Survey on Complementary and Alternative Medicine in Cancer Patients in Japan

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ABSTRACT

Purpose
To determine the prevalence of use of complementary and alternative medicine (CAM) by patients with cancer in Japan, and to compare the characteristics of CAM users and CAM nonusers.

Patients and Methods
A questionnaire on cancer CAM and the Hospital Anxiety and Depression Scale were delivered to 6,607 patients who were treated in 16 cancer centers and 40 palliative care units.

Results
There were 3,461 available replies for a response rate of 52.4%. The prevalence of CAM use was 44.6% (1,382 of 3,100) in cancer patients and 25.5% (92 of 361) in noncancer patients with benign tumors. Multiple logistic regression analysis determined that history of chemotherapy, institute (palliative care units), higher education, an altered outlook on life after cancer diagnosis, primary cancer site, and younger age were strongly associated with CAM use in cancer patients. Most of the CAM users with cancer (96.2%) used products such as mushrooms, herbs, and shark cartilage. The motivation for most CAM use was recommendation from family members or friends (77.7%) rather than personal choice (23.3%). Positive effects were experienced by 24.3% of CAM users with cancer, although all of them received conventional cancer therapy concurrently. Adverse reactions were reported by 5.3% of cancer patients. CAM products were used without sufficient information by 57.3% of users with cancer and without a consultation with a doctor by 60.7% of users.

Conclusion
This survey revealed a high prevalence of CAM use among cancer patients, without sufficient information or consultation with their physicians. Oncologists should not ignore the CAM products used by their patients because of a lack of proven efficacy and safety.

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INTRODUCTION

The WHO defines complementary and alternative medicine (CAM), or so-called traditional medicine, as follows: "a comprehensive term used to refer both to traditional medical systems such as traditional Chinese medicine, Indian ayurveda and Arabic unani medicine, and to various forms of indigenous medicine."[1] CAM therapies include medication therapies (which involve the use of herbal medicine, animal parts, and/or minerals) and nonmedication therapies carried out primarily without the use of medication (such as acupuncture or manual therapy). Populations throughout Africa, Asia, and Latin America use traditional medicine to help meet their primary health care needs. In addition to being accessible and affordable, traditional medicine is also often part of a wider belief system, and is considered integral to everyday life and well-being. In Europe and North America, CAM is increasingly being used in parallel to

allopathic medicine, particularly for treating and managing chronic disease. Concerns about the adverse effects of chemical medicines, a desire for more personalized health care, and greater public access to health information fuel the increasing use of CAM in many industrialized countries.[2-5]

The widespread use of a variety of nutritional, psychological, and natural medical approaches as CAM has been well documented.[2,6-8] Recent surveys demonstrate that more than 50% of US cancer patients use CAM therapies at some point after their diagnosis.[3,6,7] Despite extensive use, there is a paucity of data available to indicate whether these practices are efficacious and safe.[9-11] Therefore, serious research efforts are underway to determine the scope of CAM use by patients and their motivations for its use.[6-10] CAM in cancer medicine seems to be widely available in Japan as well as in the Western countries. We performed a preliminary survey on cancer CAM in a single cancer center in 1999. This survey revealed that 32% of cancer patients used CAM, and the most frequently used CAM involved natural products, such as mushrooms, shark cartilage, and beeswax-pollen mixtures.[12] The most pressing and significant problems associated with these products were commonly held but incorrect assumptions and the absence of any regulatory oversight. In addition, interactions between herbs and drugs may increase or decrease the pharmacologic or toxicologic effects of either component. For example, St John's wort has recently been reported to dramatically reduce plasma levels of SN-38 (the active metabolite of irinotecan, a key oncologic drug), which may have a deleterious impact on treatment outcome.[13]

An enormous amount of unreliable information on cancer CAM is available from the Internet and other media sources. It is often the case that cancer patients and their relatives are at a loss about how to deal with such information and have a difficult time choosing what kind of CAM they should adopt. However, there have been no large-scale surveys of this sort in Asia, and the actual state of CAM use in cancer patients is still unclear. Therefore, we performed a nationwide cross-sectional survey to evaluate the prevalence of CAM use in cancer patients and their perceptions of cancer CAM, especially of CAM products used in Japan.

PATIENTS AND METHODS

Participants

Before initiation of this survey, the study protocol was examined by the institutional review boards of cancer centers and related hospitals (CCs) joining the nationwide association of medical centers for cancer and adult diseases in Japan, and hospice and palliative care units (PCUs) joining the Japanese association of palliative care. Sixteen of 29 CCs and 40 of 88 PCUs approved the survey. All participating institutions agreed not to treat patients systematically with any CAM. The total number of questionnaires that would be distributed to the patients was predicted by the responsible physician working for each collaborating institute, and this information was provided in advance to the National Shikoku Cancer Center. Questionnaires on cancer CAM were then sent to the responsible collaborating physicians in the CCs and PCUs from October 2001 to March 2002. The day on which the questionnaires were distributed to the patients was determined voluntarily by each institute within 2 weeks of receipt. Questionnaires were distributed to the patients by the medical staff (physicians, nurses, clerks, and so on) at each collaborating institute after exclusion of those with an Eastern Cooperative Oncology Group performance status of 4 and those who underwent surgery that day. Replies were sent back to the National Shikoku Cancer Center directly from each patient. Questionnaires were marked in advance to identify the type of clinic the patients were attending (ie, CCs or PCUs, and inpatient or outpatient). Returned questionnaires were coded with an identification number to ensure confidentiality.

Questionnaire

We had previously evaluated a questionnaire about cancer CAM in 219 cancer patients who were admitted to the National Shikoku Cancer Center as a preliminary study.[12] In the present study, we used a modified version of that questionnaire after testing several samples. Some additional questions were quoted from previously published articles.[6-8] The original questionnaire we used was written in Japanese. The attached questionnaire (Appendix) has been translated into English. The questionnaire was developed through a systematic literature review and discussions by two experienced medical oncologists, a psychiatrist, a pharmacist, a basic scientist, and a research assistant. On the cover page of the questionnaire, CAM was clearly defined as follows: "any therapy not included in the orthodox biomedical framework of care for patients. CAM means remedies that are used without the approval of the relevant government authorities, such as the Ministry of Health and Welfare in Japan, that approve new drugs after peer review of preclinical experiments and clinical trials regulated by law. CAM usually skips these steps and is offered directly to the public. Health insurance does not usually cover the cost of CAM, and patients will be liable for the whole expense incurred by any CAM. CAM includes natural products from mushrooms, herbs, green tea, shark cartilage, other special foods, megavitamins, acupuncture, aromatherapy, massage, meditation, and so on."

The questionnaire was composed of the following two parts: background of the patients (disease, onset, age, sex, daily living activity level, educational level, religion, cancer treatment, changes of outlook on life, satisfaction with receiving conventional medicine, and use of cancer CAM; questions 1 to 12) and users' perception of cancer CAM (initiation time, kinds of CAM used, reason for starting CAM, method of obtaining information about the CAM used, expectations for CAM use, effectiveness or ineffectiveness, adverse effects, average expense per month, whether a history of CAM use was provided to the physician in charge, whether the physician in charge was consulted, response of physician, reason for not consulting physician, and concurrent use of anticancer drugs and CAM products that are sold over the counter; questions 13 to 28).

Hospital Anxiety and Depression Scale

A brief scale, the Hospital Anxiety and Depression Scale (HADS), was used in this study to clarify the relationship between emotional state and CAM preference. The HADS has 14 items in two question groups, one each on anxiety and depression, and each question is rated from 0 to 3. The validity and reliability of the Japanese version of HADS have been confirmed previously.[14,15] From previous articles, including the original one and studies in the Japanese population, we adopted 10 points as the cutoff above which anxiety and depression would be scored as high.[14-16] The patients in the high group were considered to have an adjustment disorder or more severe condition. The HADS was delivered to patients along with the questionnaire on CAM.

Statistical Analysis

Differences of CAM use within categories of selected demographic and clinical variables (age, sex, disease sites, daily living activity level, patient's desire, changes of outlook on life, institute, education, and religion) were assessed by the χ^2 test. The factors predicting CAM use were analyzed by univariate analysis and then multiple logistic regression analysis was performed using all significant predictor variables ($P < .05$). The analysis provided an odds ratio and 95% CI for each variable while simultaneously controlling for the effects of other variables. Variables not contributing substantially to the model were systematically removed in a backward stepwise regression process using the likelihood ratio test as the criterion for removal. The Hosmer-Lemeshow χ^2 test was used to assess the goodness of fit between the observed and predicted number of outcomes for the final model, with $P > .05$ indicating a good fit. All analyses were performed using SPSS Base and Regression models 11.0J (SPSS Japan Inc, Tokyo, Japan)

RESULTS

Response Rate to Questionnaire and CAM User Rates

A total of 6,607 questionnaires on cancer CAM were sent to collaborating CCs and PCUs according to the required number estimated by the primary investigators at those institutes. As a result, questionnaires were delivered to 6,074 patients who were treated in CCs (2,688 inpatients and 3,386 outpatients) and to 533 patients who were treated in PCUs (367 inpatients and 166 outpatients). A total of 3,733 questionnaires were returned to our center, of which 3,461 were valid with useable answers. The remaining 272 returned questionnaires were invalid because of a critical lack of major answers, such as unwritten diagnosis or no response to CAM use. Consequently, the rate of valid replies was 52.4%. Of the valid replies, 3,100 were from cancer patients and 361 were from noncancer patients with benign tumors. The flow diagram of the study population is indicated in Figure 1.

The prevalence of CAM use in cancer patients was 44.6% (1,382 of 3,100) and that in noncancer patients was 25.5% (92 of 361). In terms of background differences, noncancer patients were younger, had less impaired daily activity, and were much more likely to be in CCs than cancer patients. The rate of use among cancer patients was significantly higher than that for noncancer patients ($P < .0001$). All of the 3,100 replies from cancer patients were subject to analysis. Many users (86.7%) started CAM after their diagnosis of cancer and 73.3% of users were continuing it at the time of the survey.

Backgrounds of Patients and CAM Users

The backgrounds of all the cancer patients and CAM users with cancer are summarized in Table 1. The prevalence of CAM use was significantly higher in patients who were younger than 61 years old ($P < .0001$), female ($P < .0001$), patients with a lower daily activity level ($P < .0001$), patients with higher education ($P < .0001$), patients who received chemotherapy ($P < .0001$), patients with a change of outlook on life ($P < .0001$), patients who were dissatisfied with conventional treatments ($P = .0001$), patients in PCUs ($P < .0001$), and patients with a low HADS anxiety score

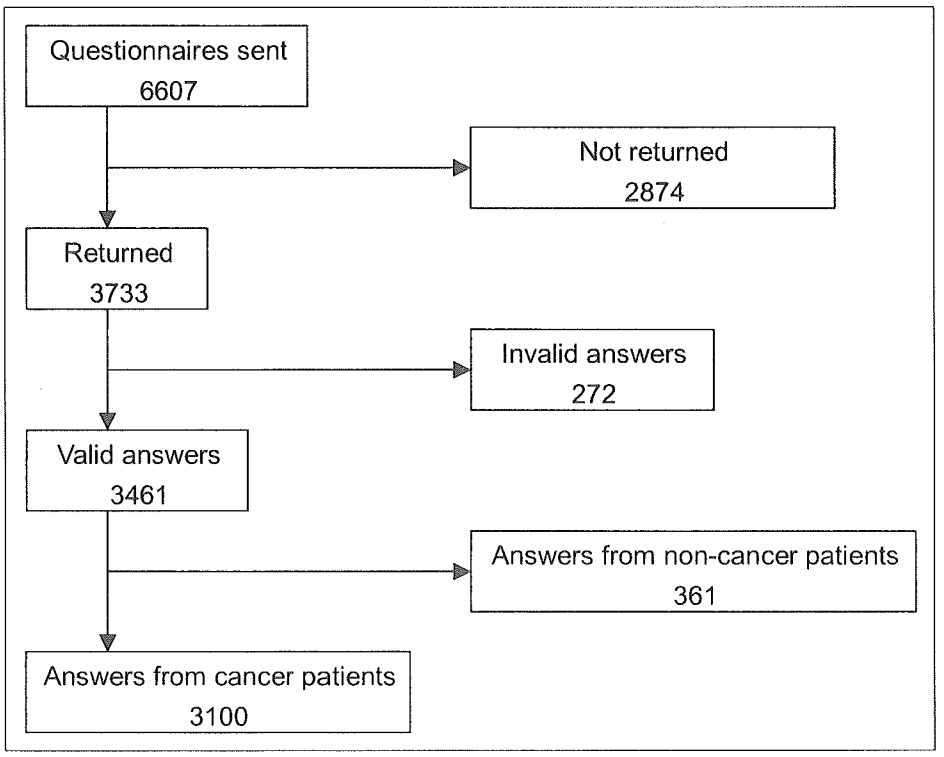

Fig 1. Flow diagram of the study population.

Table 1. Background and CAM Usage

Background	No. of Patients	No. of Users	%	P (χ^2 test)
Total	3,100	1,382	44.6	
Age, years				
> 60	1,603	625	39.0	
≤ 60	1,485	752	50.6	< .0001
Sex				
Male	1,484	586	39.5	
Female	1,614	796	49.3	< .0001
Activity of daily living				
Free or somewhat limited	2,293	1,002	43.7	
Bed rest (≥ 50% of each day)	726	348	47.9	< .0001
Education				
High school	1,721	719	41.8	
Post–high school	879	464	52.8	< .0001
Practicing religion				
No	2,140	945	44.2	
Yes	593	281	47.5	.1660
Conventional treatment				
Chemotherapy	1,839	968	52.6	
Nonchemotherapy	1,260	414	32.9	< .0001
Change in outlook on life				
No	1,381	509	36.9	
Yes	1,558	793	50.9	< .0001
Treatment met patient's needs				
No	1,212	591	48.8	
Yes	1,830	762	41.7	.0001
Institute				
Cancer centers	2,811	1,203	42.8	
Palliative care units	289	179	61.9	< .0001
Treatment place				
Inpatient ward	1,665	717	43.1	
Outpatient clinic	1,434	665	46.4	.0699
HADS				
High anxiety score (≥ 11)	1,915	852	44.5	
Low anxiety score (< 11)	741	378	51.0	.0029
High depression score (≥ 11)	1,018	510	50.0	
Low depression score (< 11)	1,652	734	44.4	.0049
Cancer				
Lung	380	203	53.4	
Breast	532	273	51.3	
Hepatobiliary	256	129	50.4	
Genitourinary	445	195	43.9	
Gastrointestinal	708	278	39.3	
Head and neck	266	82	30.8	
Other	513	222	43.3	< .0001

Abbreviations: CAM, complementary and alternative medicine; HADS, Hospital Anxiety and Depression Scale.

($P = .0029$) and a high HADS depression score ($P = .0049$). In terms of disease sites, the rate of use was higher in patients with lung, breast, and hepatobiliary cancers than in those with other cancers ($P < .0001$). The prevalence of CAM use in inpatient wards of CCs and that in outpatient clinics of CCs was 40.6% and 45.3%, respectively. The prevalence of CAM users in inpatient wards of PCUs and that in outpatient clinics of PCUs was 61.0% and 64.3%, respectively. The prevalence of CAM use in PCUs was significantly higher than that in CCs in outpatient clinics ($P < .0001$), as well as inpatient wards ($P < .0001$). Similarly, the prevalence of CAM use in inpatient wards was significantly higher than that in outpatient clinics in both CCs ($P < .0001$) and PCUs ($P < .0001$).

Predictors of Cancer CAM Use

Multivariate logistic regression analysis was performed to detect the factors predictive of CAM use, using the variables with a significantly different rate among users. The institutional review board of one CC did not permit the questions about education and religion, and 500 questionnaires in which those two questions were deleted were sent to that center. As the result, the rate of reply on education and religion was apparently low. Given that the anxiety and depression scores of HADS could not be calculated if one of each of seven questions was not answered, the number of available replies was also decreased relative to the other questions. For these reasons we performed two analyses of

the relevant variables separating the two patient populations: analysis 1 included the significant variables other than education and HADS, and analysis 2 included all significant variables as shown in Table 2. Patients who received chemotherapy; patients in PCUs; patients whose outlook on life had changed; patients with lung, breast, or hepatobiliary cancer; patients younger than 61 years old; and female patients were more likely to use CAM in both sets of analysis. In analysis 2, higher education was determined as a potent predictive factor, and dissatisfaction with conventional treatments was a weak predictive factor.

Types of CAM

The types of CAM used are listed in Table 3. The majority of CAM users (96.2%) relied on CAM products as opposed to nonmedical therapies. The most frequently used CAM product was mushrooms (Agaricus 60.6% and active hexose correlated compound [AHCC] 8.4%). Agaricus is extracted from a particular type of mushroom, *Agaricus blazei Murill.* It is purported to be an interferon inducer. AHCC is thought to act as an immunomodulator. Other CAM products were propolis (28.8%), Chinese herbs (7.1%), chitosan (7.1%), and shark cartilage (6.7%). Propolis is a beeswax-pollen mixture. Chitosan is an extract from crustaceans, such as crabs and lobsters. These are claimed to be enhancers of the immune system. Shark cartilage is known to be an inhibitor of tumor angiogenesis.[17] Chinese herbs (easily bought over the counter, but not prescribed by physicians) were used by 7.1% of patients. The rate of use of traditional Chinese medicine (qigong, moxibustion, and acupuncture) was less than 4%.

Perceptions and Attitudes Toward CAM

As shown in Table 3, 77.7% of the patients started using CAM on recommendation from family members or friends. Only 23.3% of the patients decided to use CAM on the basis of their own will. Patients expected the following effects from CAM: suppression of tumor growth (67.1%), cure (44.5%), symptom relief (27.1%), and complementary effects to conventional therapy (20.7%). In terms of the effectiveness of CAM, 24.3% of the patients experienced positive effects, such as tumor shrinkage, inhibition of tumor growth, pain relief, fewer adverse effects from anticancer drugs, and feeling better. However, at the same time, all of the patients were treated with conventional therapies such as surgery, chemotherapy, hormonal therapy, and/or radiation. The effects were not related to the use of any specific CAM product. Almost two thirds of the patients did not know if the CAM really worked or not. Conversely, only 5.3% of the patients experienced adverse effects, such as nausea, diarrhea, constipation, skin eruption, and liver dysfunction. No adverse effects were experienced by 62.2% of the patients. Patients who were uncertain about adverse effects comprised 32.6% of respondents.

More than half of the patients (57.3%) started CAM without obtaining enough information on it. Most of the patients (84.5%) had not been asked about CAM use by their physician or other health professionals. Nearly two thirds of the patients (60.7%) have never consulted their physicians on CAM use. When the patients consulted their physicians, 60.3% of the patients were told that they were free to use it or not. Patients who were told to continue using CAM and those who were told to cease use comprised 10.5% (8.5% in CCs and 19.5% in PCUs) and 11.3% (12.2% in CCs and 7.3% in PCUs) of CAM users, respectively. The main reason (56.1%) given for why they were not willing to ask their physicians about CAM was that their physicians did not ask about CAM use. The prevalence of patients who thought the physicians would not understand CAM and who thought they would prohibit CAM use was 19.4% and 8.7%, respectively.

The prevalence of concurrent use of anticancer drugs and CAM products was 61.8% in CAM users. The average monthly expenditure for CAM was 57,000 yen (approximately US $500; range, 0 to 1200,000 yen).

Table 2. Analysis of CAM Use With Multivariate Logistic Regression

Variable (reference)	Analysis 1 (n = 2,810)*			Analysis 2 (n = 2,020)†		
	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*
Used chemotherapy (*v* did not)	2.06	1.75 to 2.43	< .0001	2.24	1.85 to 2.73	< .0001
Seen at a palliative care unit (*v* a cancer center)	2.29	1.73 to 3.03	< .0001	2.22	1.59 to 3.10	< .0001
Experienced a change in outlook on life (*v* did not)	1.47	1.25 to 1.73	< .0001	1.40	1.15 to 1.70	.0007
Lung, breast, hepatobiliary cancer (*v* other cancers)	1.47	1.25 to 1.73	< .0001	1.34	1.10 to 1.62	.0031
≤ 60 years of age (*v* > 60 years)	1.39	1.18 to 1.64	< .0001	1.32	1.08 to 1.61	.0063
Symptomatic (*v* asymptomatic)	1.16	0.98 to 1.36	.074	1.23	1.01 to 1.49	.0373
Did not meet patient's needs (*v* met them)	1.21	1.03 to 1.42	.0234	1.22	1.00 to 1.48	.047
Female (*v* male)	1.17	0.98 to 1.40	.0764	1.16	0.94 to 1.43	.174
More educated (*v* less educated)	—	—	—	1.61	1.32 to 1.95	< .0001
Low HADS score for anxiety (*v* high score)	—	—	—	1.11	0.90 to 1.38	.3227
High HADS score for depression (*v* low score)	—	—	—	1.02	0.84 to 1.25	.8447

Abbreviation: HADS, Hospital Anxiety and Depression Scale.
*Analysis 1 was performed with all variables except for education and HADS because there were fewer responses for these variables.
†Analysis 2 was performed with all variables listed.

Table 3. Types of CAM Used and Perceptions and Attitudes of 1,382 CAM Users

Characteristic	%
Type of CAM used*	
CAM products (Chinese herbs, mushrooms, shark cartilage, vitamins, and so on)	96.2
Qigong†	3.8
Moxibustion	3.7
Acupuncture	3.6
Motive for starting CAM	
Recommendation from family or friends	77.7
Will of patients themselves	23.3
Expectations for CAM use*	
Suppress cancer growth	67.1
Cure	44.5
Symptom relief	27.1
Complementary effects to conventional therapy	20.7
Positive effects	
Yes	24.3
No	6.2
Unclear	69.5
Adverse effects	
Yes	5.3
No	62.2
Unclear	32.6
Obtained enough information on CAM	
Yes	42.7
No	57.3
Heard about CAM use from health professionals	
Yes	15.5
No	84.5
Consulted with doctors about CAM use	
Yes	39.3
No	60.7

NOTE. Unanswered rates were less than 10% in all categories.
*Questions in which multiple selections of answers were allowed.
†Component of traditional Chinese medicine that combines movement, meditation, and regulation of breathing to enhance the flow of vital energy (qi) in the body to improve circulation and enhance immune function.

DISCUSSION

The surveyed cancer population in this study used complementary but not alternative therapies because they were simultaneously treated in conventional medical facilities. However, we could not completely rule out the possibility that they had previously used alternative medicine. Therefore, we used the term CAM in this study.

Although we received more than 3,000 replies, the response rate (52.4%) was a little lower than in previous studies.[3,6,18,19] This may have introduced bias into our study. However, the patients' privacy was completely preserved and our survey method was the easiest way for the patients to reply to the questionnaire without feeling any pressure. We believe that our survey is helpful for assessing regional research priorities and for comparing the current status of CAM use in studies using a similar mailed-questionnaire method in other countries.

The prevalence of CAM use in cancer patients was significantly higher than that in noncancer patients. Most of the noncancer patients in this study had benign tumors and attended the cancer centers. Therefore, the noncancer patients in our study represent neither the general healthy population nor patients with benign chronic disease. Indeed, the rate of CAM use in the general population of people suffering from disease in our country was reported to be higher than that of our noncancer patients.[20] The prevalence of CAM use in cancer patients was 44.6%. This rate was slightly higher than that found in our previous study (32%) of a single cancer center survey.[12] The prevalence appears to increase each year in our country, as in the Western countries.[2] CAM user rates were significantly higher in patients undergoing chemotherapy and in patients in PCUs, and these associations were confirmed by multivariate analysis. Chemotherapy is usually delivered to inoperable, advanced, or metastatic cancers with a palliative intent but not a curative intent. In PCUs, there were no conventional treatments with tumor shrinkage as the expected outcome. Patients' relatives or friends often recommended that the patient use CAM products in that situation. In general, medical professionals in PCUs are rather generous in accepting the use of CAM. The percentage of patients whose CAM use had been recommended was approximately two-fold higher in PCUs (19.5%) compared with that in CCs (8.5%). These are probably the primary reasons for the high rate of CAM use in patients undergoing chemotherapy and in PCUs. The multivariate analysis also revealed a close association between CAM use and high educational status, changes in outlook on life, primary cancer site, and younger age. The patients' perception of received conventional treatments and female sex were marginal predictors in our study. Predictors of CAM use have been reported in many previous studies,[7,8,19] and our data support that these predictors are similar to those in developed countries. With few exceptions, the literature indicates that highly educated patients and younger patients tend to use CAM.

Different predictors are associated with the different types of CAM used. In our surveyed population, the most frequently used CAM was natural products. Oral intake of medications is more likely in patients with lung, breast, and hepatobiliary cancers than in patients with head and neck, GI, and urogenital cancers, taking the sites of disease and the manners of progression into consideration. This is likely to be closely related to the use of CAM products because all of these are oral supplements. The predictors chemotherapy and disease site would therefore be related to the type of CAM used (ie, CAM products). Indeed, this hypothesis was suggested in a previous report in which predictors shifted to include chemotherapy after spirituality and psychotherapy or support groups were excluded from the types of CAM used.[7] Supplements (herbs or vitamins) were the main types of CAM used by the patients of that limited analysis. Unexpectedly, psychological factors such as anxiety and depression showed no relation to the use of CAM. However, these factors frequently fluctuate during the disease course, as we observed in the process of informed consent.[15] If the HADS had been administered when the patients initiated CAM use, the results would likely be different.

The majority of CAM users in this study took products such as mushrooms, herbs, and shark cartilage. Mushrooms (Agaricus and AHCC) were the most frequently used among the products. This was characteristic of our CAM users. The popular types of CAM in Western countries, such as spiritual practice, mind and body therapy, vitamins and special diet, and homeopathy, were rarely used in our country. Such mushrooms are sold in Japan as diet supplements. The providers emphasize their effects on boosting the immune system based on basic experimental findings using cultured human tumor cells, and advertise in many magazines or through the Internet with anecdotal reports of users. No reliable, well-designed clinical trials in cancer patients have been performed with these mushrooms. Nonetheless, many cancer patients used such products hoping for tumor growth suppression (67.1%) and cure (44.5%) rather than complementary effects (20.7%). These mushrooms and other similar natural products are generally expensive. This contributed to the high expenditure on CAM among our users (US $500 per month on average), compared with that in the Western countries (US $50 to $70 per month on average).[6] The main motive for CAM use was the recommendation of family members or friends. The population of patients who were willing to seek out CAM on their own was unexpectedly small, about one fourth of the users. It has been reported that support group dynamics influence individuals to be more likely to use CAM among breast cancer survivors.[6] In our study, many patients seemed to be motivated to use CAM by the recommendations of relatives. Friends also offered recommendations on CAM use.

Approximately one fourth of the users experienced positive effects from CAM, even though they all received conventional therapies previously or concurrently. Although it was unclear whether the positive effects were due to the CAM products or the conventional treatments, they nonetheless believed that the CAM was effective. In retrospect, we should have added a question to our questionnaire about the effectiveness of the conventional treatments received. Conversely, most patients reported no adverse reactions to CAM. However, the potential for harmful drug–CAM product interactions exists.[21-23] Herbs or vitamins can mask or distort the effects of conventional drugs.

This survey revealed that approximately 60% of users started CAM without obtaining enough information about it, and without informing their doctors. This proportion was similar to that in our previous survey.[12] The same issues have been pointed out in many reports from the United States and Europe.[7,24,25] In our survey, when patients consulted their physicians, 60.3% of the patients were told that they were free to continue using CAM or to stop, whereas 10.5% of the patients were told to continue using CAM and 11.3% of the patients were told to stop. These figures were also similar to the results in our previous study of clinical oncologists.[26] When oncologists were asked, 74% of them neither recommended nor prohibited the use of the products. Twelve percent of them encouraged their patients to use CAM products, and 6% told their patients to stop. It appears that a difficult situation for many oncologists emerges because of the lack of scientific information on CAM. However, physicians should acknowledge that the main reason (56.1%) patients did not inform their physicians of their CAM use was that the physicians did not ask them about it. These results indicate that better patient-physician communication and more reliable information on CAM products are needed. The prevalence of concurrent use of anticancer drugs and CAM products was considerably high (61.8%) in the present study. In our previous survey of oncologists, 83.9% of oncologists had administered anticancer drugs concurrently with CAM products.[12] Nevertheless, our present knowledge of interactions is incomplete, especially regarding anticancer drugs.[22,23] More research is urgently needed. Oncologists should be aware of these facts, and the use of CAM products should be determined before initiating chemotherapy, especially when using new investigational drugs.

A few limitations of this study must be acknowledged. First, the response rate was somewhat low compared with that of other studies, although it was greater than 50%, as discussed previously. Second, there is no definite evidence that our study population is representative of cancer patients in Japan. It seems impossible to select cancer patients randomly from throughout the entire country. We used the associations of CCs and PCUs in Japan as our survey source. Otherwise, such a large-scale survey could not be performed. These limitations have also been reported in the previous literature,[7,8] and unfortunately, inconsistencies in measures of CAM and differing patient populations and methodologies (ie, interviews *v* mailed surveys) limit the generalization of studies on CAM use.[3,4] Third, two questions were deleted from the questionnaire sent to one of the CCs. As a result, about 500 replies on education and religion were lacking. However, the analyses with or without the data from that center achieved similar results. Therefore, this did not significantly affect our conclusions.

Many cancer patients continue receiving oncologic care with standard therapies while pursuing CAM methods. A recent survey regarding the impact of the media and the Internet on cancer patients revealed that 71% of cancer patients actively searched for information, and 50% used the Internet.[27] The survey concluded that strategic efforts were needed to provide guidance for patients to help them better interpret such medical information. Oncologists need to be aware of the importance of this issue and of the rationale used to promote CAM. A great need for public and professional education regarding this subject is evident.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.