Intercellular centrosome number is correlated with the copy number of chromosomes in bladder cancer

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Abstract

Centrosome amplification, which may accelerate tumor progression through chromosomal instability, is frequently observed in human malignancies. However, the intercellular relation between the number of centrosomes and chromosomes is poorly understood. Therefore, the relationship between centrosomes and chromosomal copy number in the same cells was investigated in bladder cancer. Centrosomes were evaluated by immunohistochemistry using anti-γ-tubulin antibody in 8 bladder cancer cell lines. Fluorescence in situ hybridization with centromeric probes for chromosomes 7, 9 and 17 was then performed on the same cells stained with γ-tubulin. The number of centrosomes was directly proportional to the number of chromosomes in cells with centrosome amplification, while a large intercellular variation in chromosomal copy number was detected in cells with normal numbers of centrosomes. Cancer cells with centrosome amplification of even centrosome numbers had significantly more even numbers of chromosomes. In cancer cells with 4 centrosomes, even numbers of chromosomes were more frequently detected (87.5%). These bladder cancer cell lines showed Aurora-A and p53 overexpression. These data indicate the occurrence of centrosome amplification with the possible mechanism of cytokinesis failure, resulting in a doubling of the number of centrosomes and chromosomes.

Introduction

The centrosome is a major microtubule organizing center for the formation of bipolar mitotic spindles, and plays an important role in accurate chromosome segregation to daughter cells. Accumulating evidence has suggested that centrosome amplification, which leads to the formation of multipolar spindles and unequal segregation of chromosomes, is both a common and major factor for chromosomal instability (CIN) [1] [2] in several human malignancies [3-5]. Previous studies have reported that centrosome amplification is closely related not only to the tumor grade and DNA ploidy but also to CIN in bladder cancer [6-8].

There are several plausible mechanisms for centrosome amplification: uncoupling of centrosome duplication from cell cycle progression, centrosome multiplication following DNA damage [9] and failure of cytokinesis [10] by abnormalities of several mitotic kinases, including Aurora-A [11]. Mutational inactivation of tumor suppressor proteins such as p53 has been reported to cause centrosome amplification by multiple mechanisms [2, 12]. If centrosomes duplicate more than once in a single cell cycle, the number of centrosomes can be either odd or even. If cells fail to undergo cytokinesis, resulting in a doubling of the number of centrosomes and chromosomes, the number of centrosomes should always be even. Centrosome amplification occurs in most cases through both deregulated duplication of centrosomes and cytokinesis failure [2].

We previously, demonstrated that KK47 bladder cancer cells show a normal number (one or two) of centrosomes with disomy (two copies) of chromosomes 7 and 9 in the same cell, while TCC-sup bladder cancer cells show centrosome amplification associated with numerical aberrations of chromosomes 7 and 9 in the same cell [7]. However, the intercellular relation between the number of centrosomes and

chromosomes is poorly understood. The aim of this study was to explore the relationship between centrosomes and the number of chromosome copies in the same cells in bladder cancer.

Materials and methods

Cell culture specimens

Eight established human bladder cancer cell lines (KK47, RT-4, T24, EJ-1, 5637, J82, SCaBER and TCC-sup) were used in this study. One $\times 10^4$ cells were seeded on chamber slides (4.0 cm^2) (Nunc, Naperville, USA) and cultured in RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Each cell line was plated at the same density and in the exponential phase (days 4-5) and immunohistochemical and fluorescence in situ hybridization (FISH) analysis were carried out.

Immunohistochemistry

To determine the number of centrosomes, cells were subjected to immunostaining of centrosomes using a mouse monoclonal anti-γ-tubulin antibody (Sigma, St. Louis, MO, USA), as described previously [7, 13]. The antibody–antigen complexes were detected with an Alexa 488- or 568-conjugated goat anti-mouse IgG antibody (Molecular Probes, Eugene, OR, USA) and then counter stained with 4'-diamidino 2-phenylindole (DAPI). Cells were examined under an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with triple bandpass filter sets (DAPI/Spectrum Green/Spectrum Orange). At least 100 nuclei were photographed using a digital camera (DP-70, Olympus) at 40× magnification. The number of centrosome signals in each cell was determined by observing at 1,000× magnification, and then the number of centrosomes was written on the photograph of nuclei. Centrosome amplification was defined as 3 or more centrosomes per cell [13].

Immunostaining of Aurora-A and p53 protein was performed as described previously [7, 8].

Fluorescence in situ hybridization

To explore the relationship between centrosomes and chromosomal copy number in the same cells, multi-color FISH techniques were performed, as previously described [7]. The fixation, hybridization and post-hybridization procedures of the cells, which were stained with γ-tubulin, were performed according to protocols recommended by the supplier. Chromosome numbers 7, 9 and 17 were detected using the DNA probes CEP 7, CEP 9 and CEP 17 (Vysis, Downers Grove, IL, USA), respectively. Cells were examined under an epifluorescence microscope equipped with triple bandpass filter sets (DAPI/Spectrum Green/Spectrum Orange). Using immunohistochemical analysis, according to a photograph of the nuclei in which the numbers of centrosomes were written, the number of centromeric signals in each nucleus was determined by observing at 1,000× magnification.

Statistical analysis

Statistical analysis was performed using JMP 4.0 statistical software (SAS Institute, Cary, NC, USA). The Chi square test and linear regression analysis were used for statistical analysis. For all statistical tests, a p value of less 0.05 was considered significant.

Results

Overview

The numbers of cells that could have simultaneous evaluation of centrosomes and chromosomal copy numbers of 7, 9 and 17 in bladder cancer cells are shown in Table 1. Fractions of cells with more than 3 centrosomes per cell are also shown in Table 1. Centrosome amplification, and Aurora-A and p53 overexpression were barely present in two cell lines (KK47 and RT-4), but were more prevalent in the other 6 cell lines (T24, EJ-1, 5637, J-82, SCaBER and TCC-sup).

Relationship between centrosomes and chromosomal copy number

A representative cell line with the relationship between centrosomes and chromosomal copy number in the same cells is shown in Fig. 1. A positive correlation between centrosome number and chromosome number was found in the TCC-sup cell line (Fig. 1). Linear regression analysis of the relationship between centrosomes and chromosomal copy number is summarized in Table 2.

The KK47 cell line showed an almost normal number (one or two) of centrosomes (98.8%) with disomy (two copies) of chromosome 7 (95.7%), while monosomy of chromosome 9 was found in 26.8% of cells. Bladder cancer cells with centrosome amplification had significantly more chromosomal copy numbers than cells without amplification (data not shown), while there was a large intercellular variation in chromosomal copy number in those cells with a normal number (one or two copies) of centrosomes (Fig. 2A and B).

Number of chromosomes in bladder cancer cells with centrosome amplification

Analysis of cells with centrosome amplification was performed in 6 cell lines (T24, EJ-1, 5637, J-82, SCaBER and TCC-sup) with Aurora-A and p53 overexpression. Cancer cells with centrosome amplification with an even centrosome number had significantly more even than odd numbers of chromosome 7 and odd centrosome numbers had more odd than even numbers of chromosome 7 ($p<0.0001$). A similar tendency was shown in chromosome 17 ($p=0.0870$, Table 3), but not in chromosome 9 ($p=1.0000$, Table 3).

Of 45 bladder cancer cells with 3 centrosomes, 14 cells (31.1 %) showed 5 numbers of chromosome 7 or 17 and 11 cells (24.4%) showed 3 numbers of chromosomes 7 or 17. Of 40 bladder cancer cells with 4 centrosomes, 13 cells (32.5 %), 10 cells (25.0%) and 9 cells (22.5%) showed 4, 6 and 8 numbers of chromosomes 7 or 17, respectively (Table 4). In cancer cells with 4 centrosomes, even numbers of chromosomes were more frequently detected (87.5%).

Discussion

Centrosome amplification and Aurora-A overexpression induce CIN [14, 15], which is characterized by a large intercellular variation in chromosomal copy number [1, 8]. In this study, bladder cancer cells with centrosome amplification had greater chromosomal copy numbers than cells with normal numbers of centrosomes. These data suggest that centrosome amplification leads to an increased number of chromosomes and a large intercellular variation in chromosomal copy number.

With regard to bladder cancer cells with centrosome amplification, we found that the cells with an even number of centrosomes ($n \geq 4$) had significantly more even numbers of chromosomes than odd numbers of them. In cancer cells with 4 centrosomes, even numbers of chromosomes were more frequently detected (87.5%). These bladder cancer cell lines showed Aurora-A and p53 overexpression. One of the mechanisms of centrosome amplification is because of cells failing to undergo cytokinesis, resulting in a doubling of the number of centrosomes and chromosomes [2]. Overexpression of Aurora-A may play an important role in the occurrence of centrosome amplification, where a possible mechanism could be because of failure of cytokinesis in bladder cancer [11].

In our study, cancer cells with centrosome amplification of odd centrosome numbers had significantly more odd numbers of chromosomes than even numbers of them. The number of supernumerary centrosomes caused by deregulated duplication can be either odd or even. Mutational inactivation of p53 allows multiple rounds of centrosome duplication [2, 16]. In this study, overexpression of p53 was used for immunohistochemical detection of mutant p53. Supernumerary centrosomes of the odd numbers might have occurred by deregulated duplication; however, the reason for the

association of odd numbers between centrosomes and chromosomes is unknown. It is possible that centrosome amplification occurs through both failure of cytokinesis and deregulated duplication of centrosomes [2].

In KK47 and RT-4 cell lines without Aurora-A and p53 overexpression, cells with centrosome amplification as well as polysomy of chromosomes are rare, and might become arrested and eventually undergo cell death in a p53-dependent manner [2][11]. In bladder cancer cell lines with Aurora-A and p53 overexpression, we observed a large fraction of cells with centrosome amplification and numerical aberrations of chromosomes. Interestingly, a large intercellular variation in chromosomal copy number was also detected in those cells with a normal number of centrosomes. As a result of abnormal chromosome segregation caused by supernumerary centrosomes via formation of more than two spindle poles or pseudo-bipolar spindles [2], numerical aberration of chromosomal copy number can occur, while the centrosome number may finally become one or two.

In this study, KK47 showed an almost normal number of centrosomes, while monosomy of chromosome 9 was observed in 26.8% of cells. Chromosome 9 monosomy has been established in the oncogenesis of superficial bladder cancer [17, 18], and may occur as a result of the non-disjunction of daughter chromosomes [19]. Because of tumor progression through chromosome 9 monosomy in bladder cancer, a different outcome of chromosome 9 from other chromosomes might have resulted. We found no association between even and odd numbers of centrosomes and chromosomes in chromosome 9 (Table 3). Therefore, in our analysis of cancer cells with 3 or 4 centrosomes, we excluded data of chromosome 9.

To the best of our knowledge, this is the first report to explore the relationship between centrosome and chromosome copy number in the same cells, here in bladder cancer.

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Figure legends

Figure 1. Relationship between centrosome and chromosomal copy number

Figure 2. Relationship between centrosomes and chromosomal copy number in the same cells in representative cell lines

Bladder cancer cells with centrosome amplification had more chromosomal copy numbers than cells without amplification, while a large intercellular variation in chromosomal copy number was detected in those cells with a normal number (one or two) of centrosomes (Fig. 2A and B).

Table 1. Overview of this study

Table 2. Relationship between centrosomes and chromosomal copy number in the same bladder cancer cells

Table 3. Relationship between odd/even numbers of centrosomes (n≥3) and odd/even numbers of chromosomes in bladder cancer cells

Table 4. Number of chromosome 7 or 17 in bladder cancer cells with 3 or 4 centrosomes

Table 1
Overview of this study

Cell line	Cells for simultaneous evaluation[a] (Copy number of chromosomes: cells) Chromosome 7	Chromosome 9	Chromosome 17	Centrosome amplification (%)[c]
KK47	1: 7, 2: 379, 3: 5, 4: 5, ≥5:0	1: 19, 2: 48, 3: 3, 4: 0, ≥5: 1	1: 0, 2: 1, 3: 17, 4: 0, ≥5: 0	1.21
RT-4	1: 0, 2: 1, 3: 22, 4: 82, ≥5: 3	NE[b]	1: 0, 2: 4, 3: 19, 4: 271, ≥5: 1	0.74
T24	1: 0, 2: 26, 3: 289, 4: 14, ≥5: 15	NE	1: 0, 2: 0, 3: 73, 4: 248, ≥5: 13	6.79
EJ-1	1: 0, 2: 0, 3: 5, 4: 39, ≥5: 5	1: 0, 2: 1, 3: 5, 4: 37, ≥5: 6	NE	6.12
5637	1: 0, 2: 2, 3: 29, 4: 145, ≥5: 19	NE	1: 0, 2: 3, 3: 18, 4: 42, ≥5: 3	8.81
J-82	1: 0, 2: 16, 3: 60, 4: 4, ≥5: 3	1: 0, 2: 29, 3: 45, 4: 5, ≥5: 4	NE	9.64
SCaBER	1: 0, 2: 0, 3: 16, 4: 2, ≥5: 67	1: 0, 2: 0, 3: 0, 4: 7, ≥5: 78	NE	14.1
TCC-sup	1: 0, 2: 0, 3: 0, 4: 3, ≥5: 62	1: 0, 2: 0, 3: 2, 4: 39, ≥5: 24	1: 0, 2: 1, 3: 1, 4: 4, ≥5: 46	15.4

[a] Cells that could have simultaneous evaluation of centrosome and chromosome copy number

[b] NE: could not be evaluated.

[c] Fraction of cells with more than 3 centrosomes per cell

Table 2
Relationship between centrosomes and chromosomal copy number in the same bladder cancer cells

Chromosome (Chr.) vs centrosome (Cen.)[a]									
Cell line	Chromosome 7			Chromosome 9			Chromosome 17		
KK47	Chr.=1.603+0.220×Cen.	R^2=0.109	p<0.0001	Chr.=0.966+0.447×Cen.	R^2=0.058	p=0.043	Chr.=3.091-0.091×Cen.	not calculated	
RT-4	Chr.=3.278+0.285×Cen.	R^2=0.045	p=0.284	NE[b]			Chr.=3.657+0.141×Cen.	R^2=0.020	p=0.014
T24	Chr.=1.264+0.898×Cen.	R^2=0.830	p<0.0001	NE			Chr.=2.760+0.554×Cen.	R^2=0.222	p<0.0001
EJ-1	Chr.=1.752+1.118×Cen.	R^2=0.348	p<0.0001	Chr.=1.349+1.329×Cen.	R^2=0.259	p<0.0001	NE		
5637	Chr.=2.631+0.692×Cen.	R^2=0.174	p<0.0001	NE			Chr.=2.055+0.850×Cen.	R^2=0.243	p<0.0001
J-82	Chr.=1.341+0.775×Cen.	R^2=0.310	p<0.0001	Chr.=0.838+0.947×Cen.	R^2=0.408	p<0.0001	NE		
SCaBER	Chr.=2.377+1.430×Cen.	R^2=0.543	p<0.0001	Chr.=3.450+2.694×Cen.	R^2=0.549	p<0.0001	NE		
TCC-sup	Chr.=3.355+1.113×Cen.	R^2=0.778	p<0.0001	Chr.=1.933+1.222×Cen.	R^2=0.697	p<0.0001	Chr.=1.096+2.287×Cen.	R^2=0.729	p<0.0001

[a] Linear regression analysis was used for statistical analysis.

[b] NE: could not be evaluated.

Table 3
Relationship between odd/even numbers of centrosomes (n≥3) and odd/even numbers of chromosomes in bladder cancer cells

		Centrosome (n≥3)		
	Category	Odd	Even	*p* value
Number of chromosome 7	Odd	27	12	<0.0001
	Even	6	33	
Number of chromosome 9	Odd	3	5	1.0000
	Even	8	14	
Number of chromosome 17	Odd	10	7	0.0870
	Even	4	11	

Table 4
Number of chromosome 7 or 17 in bladder cancer cells with 3 or 4 centrosomes

3 centrosomes			4 centrosomes		
Number of chromosome 7 or 17	Cells	(%)	Number of chromosome 7 or 17	Cells	(%)
3	11	24.4	4	13	32.5
4	8	17.8	5	4	10.0
5	14	31.1	6	10	25.0
6	5	11.1	7	1	2.5
7	3	6.7	8	9	22.5
8	1	2.2	10	1	2.5
9	2	4.4	12	1	2.5
11	1	2.2	14	1	2.5
Total	45		Total	40	

Fig. 1

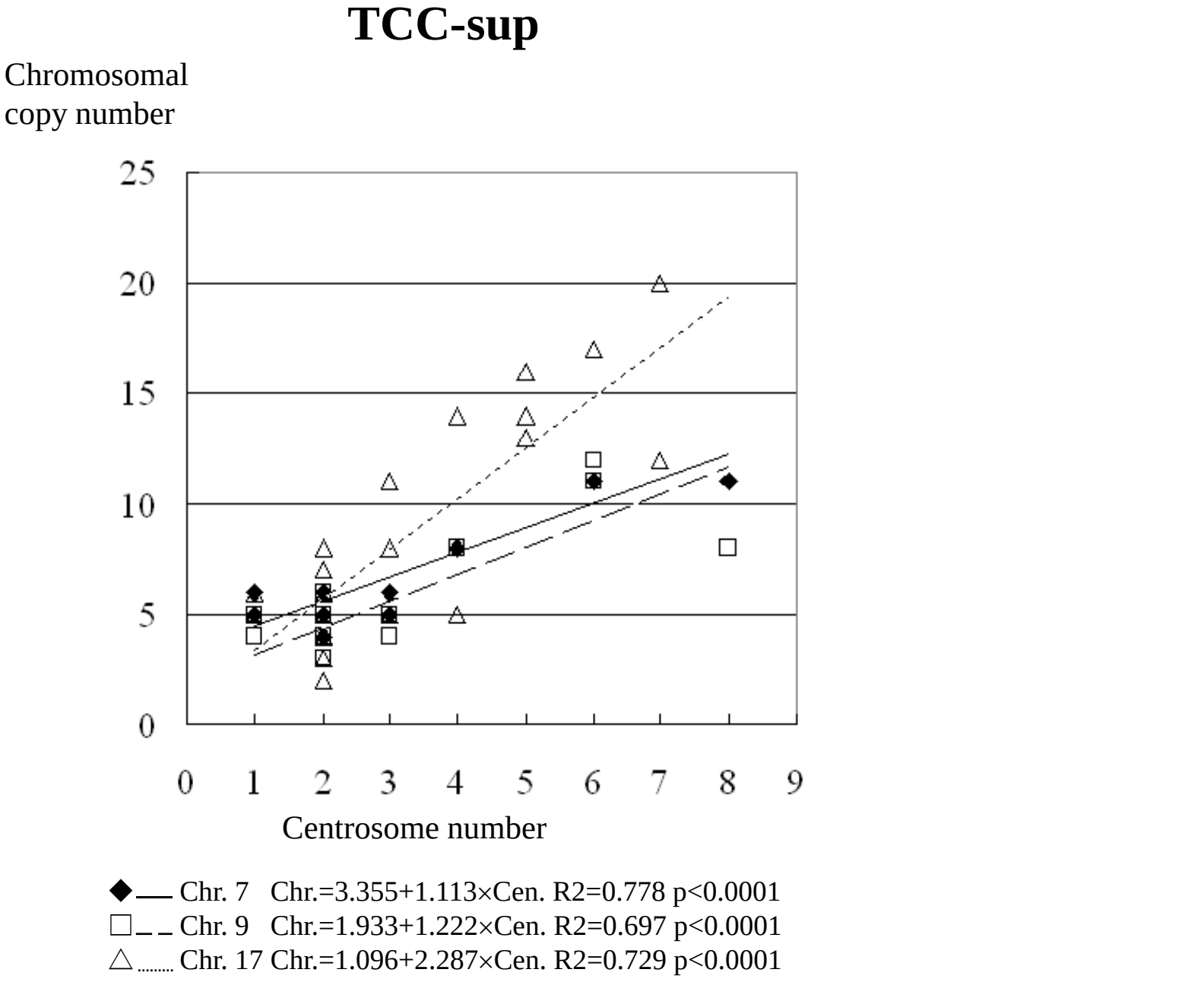
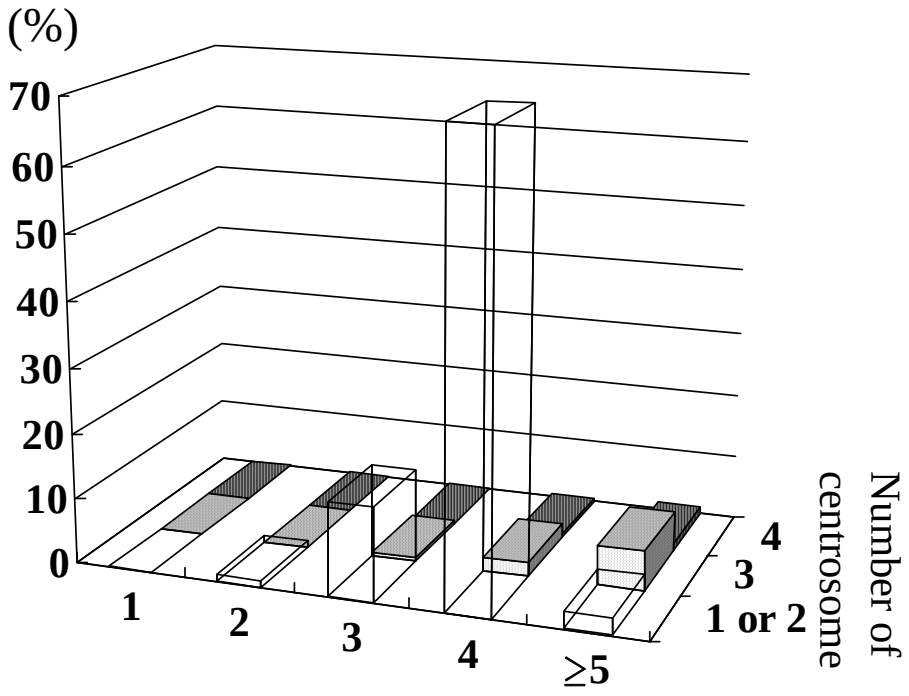

TCC-sup
Chromosomal copy number
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9
Centrosome number
Chr. 7 Chr.=3.355+1.113×Cen. R2=0.778 p<0.0001
Chr. 9 Chr.=1.933+1.222×Cen. R2=0.697 p<0.0001
Chr. 17 Chr.=1.096+2.287×Cen. R2=0.729 p<0.0001

Fig. 2

A

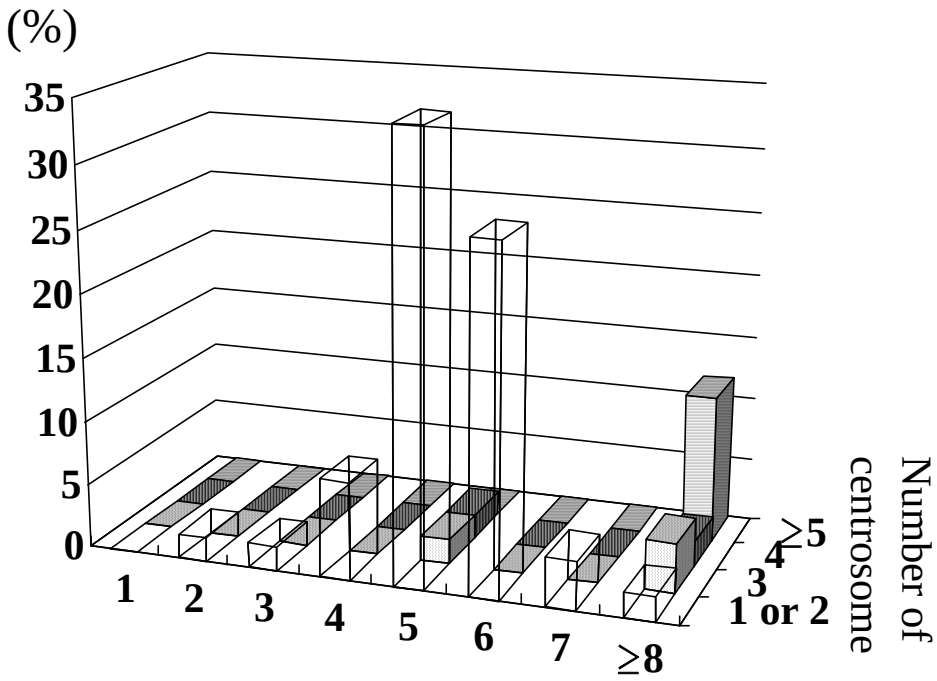
5637



Number of chromosome 7

B

TCC-sup



Number of chromosome 17