

Different chromosome damage in lymphocytes of newly diagnosed gastrointestinal and breast cancer patients

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Structural chromosome aberrations are a predictive biomarker of cancer risk. Conventional chromosome analysis widely used for these purposes detects unstable chromosome aberrations that are eliminated during cell division. Stable aberrations that may persist in the body and tend to accumulate during a lifetime can be detected by fluorescence in situ hybridization (FISH). The aim of the study was to investigate the level of chromosome damage in newly diagnosed cancer patients and control subjects by FISH. Both groups of untreated cancer patients had increased frequency of aberrant cells. However, chromosome damage affected different cytogenetic endpoints. Stable translocations and cells with complex rearrangements were elevated in breast cancer patients whereas unstable chromosome aberrations (dicentric chromosomes and acentric fragments) were elevated in gastrointestinal cancer patients. These associations observed in nonsmokers were typically not pronounced in smokers (with the exception of dicentric chromosomes in gastrointestinal patients). Exposure to tobacco smoke increased aberrations in healthy controls but not in the cancer patients. Our study suggests an association between cancer and stable chromosomal rearrangements in breast cancer patients. Unstable aberrations elevated in gastrointestinal cancer patients may be at least partly ascribed to the exposure to diagnostic X-rays.

Key words: breast cancer, gastrointestinal cancer, risk assessment, chromosome aberration, fluorescence in situ hybridization

Genomic instability, including changes in chromosome number and structure, is a typical feature of neoplastic cells. Therefore, using chromosome abnormalities as a possible biomarker to indicate cancer risk was verified by many studies. It has been shown that increased frequency of chromosome damage in healthy individuals indicates an increased cancer risk with the strongest association found for stomach cancer [1–3]. Several studies also investigated chromosome aberrations in newly diagnosed, untreated cancer patients. Association with tumor development was suggested in lung, breast, and colorectal cancer, as well as Hodgkin's lymphoma [4–8].

Of the cytogenetic endpoints, structural chromosome aberrations are the most robust predictive biomarker of cancer risk [9]. Conventional chromosome analysis using Giemsa stained metaphase chromosomes is widely used for these purposes. This method detects unstable chromosome aberrations, such as chromosome or chromatid breaks and dicentric chromosomes. However, the number of unstable aberrations decreases with time, as they are lost through cell divisions [10]. Thus, it mostly exposes recently occur-

ring chromosome damage. Scoring of dicentric chromosomes is suitable for biological dosimetry of recent and acute exposures to ionizing radiation [11, 12].

In addition to unstable chromosome aberrations, the fluorescence *in situ* hybridization (FISH) technique using painting probes is also able to detect chromosome aberrations that can survive mitotic division. Stable aberrations may persist in the body for years and tend to accumulate during a lifetime. Therefore, translocation analysis is used as a biomarker for evaluating the received doses of ionizing radiation from past or chronic exposure [13]. FISH seems to be more sensitive than conventional chromosome analysis as was shown in medical personnel and in the study of environmental air pollution impact [14–17]. Nevertheless, only a limited number of studies have used FISH to study the association between chromosome aberrations and cancer risk.

Using FISH with painting probes, we investigated the level of chromosome damage in peripheral blood lymphocytes taken from a group of patients who were newly diagnosed

with gastrointestinal and breast cancer compared to healthy control subjects. The aim of our study was to validate chromosome aberrations detected by FISH as a predictive biomarker of cancer risk.

Patients and methods

Subjects (participants). In total, we investigated 124 patients with newly diagnosed solid tumors from Masaryk Memorial Cancer Institute, Brno (MMCI). The group comprised 27 patients (16 males and 11 females; 3 current smokers, 11 former smokers and 13 nonsmokers) with gastrointestinal cancer (cancer of the esophagus, stomach, intestine, colon, rectum, liver and pancreas) and 97 female patients with breast cancer (14 current smokers, 16 former smokers and 67 nonsmokers). According to the TNM (tumor, node, metastasis) system, gastrointestinal cancer patients were in stages: I (8), II (8), III (8) and IV (3). Most of the breast cancer patients were in stages I (54) and II (40), only 2 patients were in stage III and 1 patient was not classified. Blood samples were taken from the patients prior to cancer treatment (radiotherapy or chemotherapy). A group of 79 healthy control subjects (37 males and 42 females; 8 current smokers, 27 former smokers and 44 nonsmokers) was recruited through the outpatient clinic of preventive oncology at the MMCI who have not suffered from cancer and live and work in a nonhazardous environment.

All participants provided informed consent for their participation in the study and completed a questionnaire. Information about lifestyle, potential occupational exposure, other diseases, and their treatments were collected. All acquired data and blood samples were coded and processed anonymously.

Culture of blood. Cultures from samples of heparinized venous blood were established in complete Chromosome P Medium (Euroclone, Pero, Italy) and incubated at 37°C for 48 h. Colcemid (Sigma-Aldrich, St. Louis, MO, USA) was added to a final concentration of 0.1 µg/ml 2 h before the end of incubation. The culture harvest was made using a 20 min hypotonic treatment in 0.075 M KCl prewarmed to 37°C and (3:1) methanol : acetic acid fixation. Metaphase chromosome spreads were prepared by dropping the cell suspensions onto moistened microscopic slides, which were then air-dried.

FISH. Painting probes for chromosomes 1, 2 and 4 were used because of their ability to visualize a large fraction of genome. Prior to hybridization, the locations of DAPI (4', 6-diamidino-2-phenylindole) stained metaphase cells, potentially suitable for further analysis, were established by an automatic metaphase finder system (Metafer) and the coordinates were saved. The slides were denatured in 0.07 M NaOH (according to a protocol provided by MetaSystems for multi-color probe kits) and hybridized with denatured painting probes for chromosomes 1 (orange), 2 (green) and 4 (green; MetaSystems) and a pancentromeric probe (biotin; Cambio). After overnight hybridization at 37°C,

the slides were washed in 0.4× SSC at 72°C and biotin labeled probe was detected by avidin conjugated to the near infrared fluorescent dye Cy5. Chromosomes were counterstained with DAPI. The metaphase spreads were relocated by Metafer, and 1000 cells from each sample were scored under a fluorescence microscope equipped with dual band excitation filter set for green and orange fluorescence and filters for single dyes including DAPI and Cy5. Aberrant or potentially aberrant cells were captured and classified by two independent evaluators using image analysis software ISIS (MetaSystems) according to the Protocol for Aberration Identification and Nomenclature [18]. Reciprocal translocations, the total number of translocations (translocated chromosomes), dicentric chromosomes, acentric fragments, and cells with complex rearrangements were evaluated. To calculate whole genome translocation frequency each complete reciprocal translocation (2-way translocations) and incomplete translocation (1-way translocations) were considered as one event. Apparently stable complex exchanges (3 or more breaks in 2 or more chromosomes) were broken down into simple translocations and included in the total translocation count [19, 20]. Genomic frequencies of stable chromosomal translocations (F_G) were calculated according to a standard formula proposed by Lucas and Sachs [21].

Statistical evaluation of data. Data were analyzed using the statistical program R version 3.3.3 (<https://www.R-project.org>). All tests were performed at a significance level of 5%. The Mann-Whitney test was used to compare distributions of individual parameters between groups. To control the confounding effects of age and smoking, binary logistic regression was used and adjusted odds ratios (aOR) with 95% confidence intervals were calculated.

Results

Using FISH, we investigated chromosomal aberrations in 124 patients with newly diagnosed solid tumors (median age 62.6 years) and 79 healthy control individuals (median age 54.2 years). The group of patients included 27 with gastrointestinal cancer (median age 63.4 years) and 97 with breast cancer (median age 62.3 years). The detailed results of the cytogenetic investigation are summarized in Table 1. Higher frequencies of aberrant cells were observed in gastrointestinal cancer (median 14/1000 cells, $p=0.001$) and breast cancer (median 10/1000 cells, $p=0.026$) patients than in controls (median 8/1000 cells). However, the groups of gastrointestinal cancer and breast cancer patients were older than controls ($p=0.009$ and $p=0.007$, respectively) and the proportion of smokers in patients differed from the proportion in controls. Therefore, the association between cytogenetic damage and cancer development (expressed as odds ratios) was adjusted for two main confounders – age and smoking. The results are summarized in Table 2. The analysis revealed significantly increased frequencies of aberrant cells (aOR=1.16, $p=0.009$), dicentric chromosomes (aOR=2.70,

Table 1. Chromosomal aberrations in newly diagnosed cancer patients and controls.

		Cancer patients			Controls N=79
		Gastrointestinal N=27	Breast N=97	Total N=124	
Age (years)	mean ± SD	61.9±9.8	60.4±12.3	60.7±11.8	54.8±12.9
	median	63.4	62.3	62.6	54.2
	first quartile	54.8	51.0	51.5	45.5
	third quartile	69.0	68.6	68.8	65.8
Ab.c.	mean ± SD	13.0±6.0	10.3±5.1	10.9±5.4	8.7±4.4
	median	14	10	10.5	8
	first quartile	7	7	7	5
	third quartile	17	14	14	11
rcp	mean ± SD	7.2±4.4	6.7±4.1	6.8±4.1	5.5±3.7
	median	7	5	6	5
	first quartile	4	4	4	3
	third quartile	11	9	10	8
t	mean ± SD	18.1±10.2	16.5±9.1	16.8±9.3	13.3±8.4
	median	18	14	15.5	12
	first quartile	9	9	9	7
	third quartile	25	22	23	18
dic	mean ± SD	2.1±1.4	1.0±1.2	1.3±1.3	0.8±0.9
	median	2	1	1	1
	first quartile	1	0	0	0
	third quartile	3	2	2	1
ace	mean ± SD	5.3±3.6	2.8±2.6	3.4±3.0	2.5±2.4
	median	4	2	3	2
	first quartile	3	1	1	1
	third quartile	7	4	5	3.5
complex	mean ± SD	1.3±1.1	1.6±1.4	1.5±1.3	1.0±0.9
	median	1	1	1	1
	first quartile	0.5	1	0.8	0
	third quartile	2	2	2	1
F _G /100	mean ± SD	2.49±1.38	2.25±1.24	2.30±1.27	1.84±1.12
	median	2.33	2.10	2.10	1.57
	first quartile	1.17	1.31	1.31	1.04
	third quartile	3.26	3.15	3.15	2.61

Abbreviations: Ab.c., aberrant cells/1000 cells; rcp, reciprocal translocations/1000 cells; t, total number of translocations/1000 cells; dic, dicentric chromosomes/1000 cells; ace, acentric chromosomes/1000 cells; complex, cells with complex rearrangement (three or more breaks in two or more chromosomes)/1000 cells; F_G/100, genomic frequency of stable translocations/100 cells.

$p < 0.001$) and acentric fragments (aOR=1.36, $p < 0.001$) in patients with gastrointestinal cancer in comparison with controls. The same cytogenetic endpoints were elevated when gastrointestinal cancer patients were compared with breast cancer patients (aOR=1.10, $p=0.041$; aOR=1.85, $p < 0.001$; aOR=1.30, $p=0.001$). Breast cancer patients manifested an elevated frequency of cells with complex rearrangements compared with controls (aOR=1.46, $p=0.014$). The groups of cancer patients did not differ from controls in any parameter regarding translocations.

Subsequently, subjects in the groups were divided according to their smoking habits (Tables 3 and 4) and the association of cytogenetic parameters with cancer development was evaluated in never smokers and ever smokers (current or former

smokers) separately (Table 2). In regard to nonsmokers, a significant increase of aberrant cells (aOR=1.22, $p=0.016$), dicentric chromosomes (aOR=3.80, $p=0.002$), and acentric fragments (aOR=1.60, $p=0.002$) were pronounced in the group of patients with gastrointestinal cancer when compared with controls. Elevated frequencies of dicentric chromosomes (aOR=1.93, $p=0.004$) and acentric fragments (aOR=1.31, $p=0.007$) were also observed in nonsmokers with gastrointestinal cancer when compared to nonsmokers with breast cancer. In nonsmokers with breast cancer, aside from the elevation of cells with complex rearrangements (aOR=1.93, $p=0.006$), we also observed a significant increase in the frequency of total translocations (aOR=1.07, $p=0.035$) and genomic frequency of stable translocations (aOR=1.63,

Table 2. Odds ratios of cytogenetic parameters for cancer patients (adjusted for age and smoking).

All subjects	Gastrointestinal cancer (vs. controls)				Breast cancer (vs. controls)				All cancers (vs. controls)				Gastrointestinal cancer vs. breast cancer			
	aOR	95% CI		p-value	aOR	95% CI		p-value	aOR	95% CI		p-value	aOR	95% CI		p-value
		lower	upper			lower	upper			lower	upper			lower	upper	
Ab.c.	1.16	1.04	1.30	0.009	1.05	0.97	1.14	0.215	1.08	1.00	1.16	0.056	1.10	1.00	1.21	0.041
rcp	1.03	0.91	1.18	0.630	1.04	0.95	1.15	0.405	1.06	0.95	1.14	0.387	1.01	0.90	1.13	0.875
t	1.03	0.97	1.09	0.321	1.03	0.98	1.07	0.231	1.14	0.98	1.31	0.066	1.01	0.96	1.07	0.653
dic	2.70	1.64	4.45	<0.001	1.14	0.84	1.54	0.395	1.36	1.31	1.80	0.030	1.85	1.31	2.61	<0.001
ace	1.36	1.15	1.62	<0.001	1.04	0.91	1.18	0.575	1.11	0.98	1.24	0.084	1.30	1.12	1.52	0.001
complex	1.24	0.78	1.97	0.375	1.46	1.08	1.98	0.014	1.38	1.03	1.84	0.029	0.78	0.54	1.14	0.196
F _G /100	1.25	0.81	1.93	0.314	1.23	0.90	1.69	0.199	1.24	0.92	1.66	0.160	1.10	0.75	1.60	0.635
Nonsmokers																
Ab.c.	1.22	1.04	1.43	0.016	1.14	1.01	1.27	0.033	1.14	1.03	1.27	0.014	1.07	0.96	1.20	0.219
rcp	1.12	0.92	1.36	0.272	1.12	0.97	1.30	0.111	1.12	0.98	1.27	0.097	1.01	0.87	1.17	0.889
t	1.06	0.97	1.16	0.178	1.07	1.01	1.14	0.035	1.26	1.02	1.54	0.029	1.00	0.93	1.07	0.995
dic	3.80	1.61	8.94	0.002	1.34	0.86	2.07	0.195	1.55	1.04	2.33	0.034	1.93	1.24	3.00	0.004
ace	1.60	1.19	2.14	0.002	1.12	0.93	1.35	0.230	1.19	1.00	1.42	0.050	1.31	1.08	1.59	0.007
complex	1.68	0.84	3.36	0.146	1.93	1.20	3.10	0.006	1.82	1.16	2.84	0.009	0.75	0.43	1.30	0.297
F _G /100	1.57	0.82	2.97	0.171	1.63	1.03	2.58	0.038	1.60	1.04	2.47	0.032	1.04	0.64	1.68	0.888
Smokers																
Ab.c.	1.11	0.94	1.31	0.215	0.95	0.83	1.09	0.497	1.02	0.93	1.13	0.647	1.17	0.97	1.40	0.096
rcp	0.97	0.80	1.17	0.728	0.98	0.85	1.13	0.759	1.00	0.89	1.13	0.959	1.00	0.82	1.21	0.966
t	1.01	0.93	1.09	0.894	0.98	0.92	1.05	0.613	1.01	0.96	1.06	0.848	1.03	0.94	1.13	0.526
dic	2.06	1.07	3.96	0.031	0.96	0.61	1.51	0.856	1.20	0.80	1.79	0.374	1.75	0.99	3.08	0.054
ace	1.22	0.99	1.51	0.067	0.95	0.79	1.16	0.642	1.05	0.90	1.22	0.566	1.29	1.00	1.65	0.050
complex	0.98	0.52	1.87	0.959	1.18	0.79	1.78	0.418	1.15	0.80	1.66	0.443	0.79	0.47	1.34	0.386
F _G /100	1.03	0.56	1.89	0.930	0.90	0.55	1.48	0.679	0.94	0.60	1.49	0.803	1.17	0.62	2.22	0.623

Abbreviations: Ab.c., aberrant cells/1000 cells; rcp, reciprocal translocations/1000 cells; t, total number of translocations/1000 cells; dic, dicentric chromosomes/1000 cells; ace, acentric chromosomes/1000 cells; complex, cells with complex rearrangement (three or more breaks in two or more chromosomes)/1000 cells; F_G/100, genomic frequency of stable translocations/100 cells; aOR, adjusted odds ratio; CI, confidence interval; significant values are in bold (p<0.05)

p=0.038) that was not seen when all breast cancer patients (smokers and nonsmokers) were compared with controls. In smokers, only dicentric chromosomes (aOR=2.06, p=0.031) were elevated and acentric fragments were near the statistical significance (aOR=1.22, p=0.067) in gastrointestinal cancer patients when compared to controls. No differences in cytogenetic endpoints were observed between smoker breast cancer patients and controls.

Furthermore, we compared cytogenetic parameters between smokers and nonsmokers in groups of patients and controls (Table 5). Among control subjects, smokers had significantly higher frequencies of all examined cytogenetic parameters except dicentric chromosomes. However, smoking status did not affect cytogenetic parameters in groups of newly diagnosed cancer patients.

We also compared the cytogenetic parameters with TNM stages. For statistical analysis, gastrointestinal cancer patients were divided into two groups with TNM I + II and TNM III + IV, and breast cancer patients into groups with TNM I and TNM II + III. We did not reveal an association between evaluated cytogenetic parameters and TNM stages except for unstable dicentric chromosomes in gastrointestinal cancer

patients, where higher TNM stages were associated with a lower frequency of dicentric chromosomes (aOR=0.40, p=0.033).

Discussion

Breast and colorectal cancer rank among the most frequently diagnosed cancers and are the leading causes of cancer death in developed countries [22]. In our study, we focused on chromosomal damage detected by FISH with painting probes in patients with newly diagnosed cancer and on the impact of tobacco smoke exposure on this association. As we demonstrated in nonsmokers, untreated gastrointestinal and breast cancer patients had an increased frequency of aberrant cells. However, chromosome damage affected different cytogenetic endpoints in these groups. Gastrointestinal cancer is associated with elevated frequency of unstable chromosome aberrations such as dicentric chromosomes and acentric fragments. In contrast, stable translocations and cells with complex rearrangements contributed to chromosome damage in breast cancer. Only a few studies dealing with this topic have been published. Increased levels of aberrant

Table 3. Chromosomal aberrations in newly diagnosed cancer patients and controls (nonsmokers).

Nonsmokers		Cancer patients			Controls N=44
		Gastrointestinal N=13	Breast N=67	Total N=80	
Age (years)	mean ± SD	61.5±9.1	61.2±12.2	61.2±11.7	53.1±13.4
	median	63.9	62.6	63.0	51.9
	first quartile	52.9	51.2	51.5	44.7
	third quartile	69.0	68.6	68.9	63.0
Ab.c.	mean ± SD	12.3±6.4	10.3±5.2	10.7±5.5	7.2±3.6
	median	14	10	10.5	7
	first quartile	6	7	6	4
	third quartile	16	13	14	9.25
rcp	mean±SD	6.8±5.0	6.6±4.1	6.6±4.2	4.5±2.8
	median	6	5	5	4
	first quartile	3	4	4	2
	third quartile	10	8.5	9	6
t	mean±SD	16.7±12.0	16.6±9.3	16.6±9.5	10.9±6.6
	median	18	14	14	9
	first quartile	8	9	9	5.75
	third quartile	23	22	22	14
dic	mean ± SD	2.5±1.7	1.1±1.2	1.3±1.4	0.7±0.8
	median	2	1	1	1
	first quartile	1	0	0	0
	third quartile	3	2	2	1
ace	mean ± SD	5.4±3.5	2.8±2.7	3.2±2.9	2.1±1.9
	median	4	2	3	2
	first quartile	3	1	1	0.75
	third quartile	7	4	4.2	3
complex	mean ± SD	1.2±1.1	1.5±1.3	1.5±1.3	0.8±0.8
	median	1	1	1	1
	first quartile	0	1	0.8	0
	third quartile	2	2	2	1
F _G /100	mean ± SD	2.31±1.48	2.24±1.27	2.25±1.30	1.49±0.91
	median	2.33	1.84	1.97	1.30
	first quartile	1.05	1.31	1.31	0.79
	third quartile	3.15	3.15	3.15	1.84

Abbreviations: Ab.c., aberrant cells/1000 cells; rcp, reciprocal translocations/1000 cells; t, total number of translocations/1000 cells; dic, dicentric chromosomes/1000 cells; ace, acentric chromosomes/1000 cells; complex, cells with complex rearrangement (three or more breaks in two or more chromosomes)/1000 cells; F_G/100, genomic frequency of stable translocations/100 cells.

cells with unstable chromosome aberrations detected by conventional analysis using Giemsa stained chromosomes were found in untreated breast, prostate, uterus/ovary, head/neck, bladder/kidney and lung cancer patients [7, 23]. As the authors did not observe increased levels of typical postradiation aberrations (i.e., dicentric chromosomes), they concluded that more frequent diagnostic X-ray examination during diagnosis in these patients did not have a critical effect. Another study showed an increase in conventional cytogenetic endpoints including dicentric chromosomes in lung cancer patients [8]. In our study, the level of dicentric chromosomes in gastrointestinal cancer patients was elevated in both nonsmokers and smokers. Thus, it

cannot be ruled out that acute exposure to diagnostic X-rays might have contributed to the increase of unstable aberrations found here. No elevation of the frequency of dicentric chromosomes was observed in breast cancer patients. In fact, gastrointestinal cancer patients receive greater radiation doses during diagnostic examinations compared to breast cancer patients (median 29.83 mSv vs. 0.35 mSv, unpublished results of MMCI) because they routinely undergo computed tomography scans.

Contrary to findings in gastrointestinal cancer patients, an increased level of stable translocations was observed in breast cancer patients (nonsmokers). Our finding is in agreement with the study by Verdorfer *et al.* [4] who investigated

Table 4. Chromosomal aberrations in newly diagnosed cancer patients and controls (smokers).

Smokers		Cancer patients			Controls N=35 (8/27)*
		Gastrointestinal N=14 (3/11)*	Breast N=30 (14/16)*	Total N=44 (17/27)*	
Age (years)	mean \pm SD	62.3 \pm 10.7	58.6 \pm 12.6	59.8 \pm 12.0	56.8 \pm 12.1
	median	63.3	60.7	62.5	60.2
	first quartile	57.1	50.8	51.5	50.0
	third quartile	68.1	68.3	68.7	66.3
Ab.c.	mean \pm SD	13.6 \pm 5.8	10.3 \pm 4.7	11.4 \pm 5.2	10.5 \pm 4.6
	median	14	9.5	10.5	10
	first quartile	10.25	7.25	8	7.5
	third quartile	17	14	15.3	13.5
rcp	mean \pm SD	7.6 \pm 4.0	7.0 \pm 4.1	7.2 \pm 4.0	6.9 \pm 4.3
	median	7.5	6	6.5	7
	first quartile	4.25	4	4	4
	third quartile	11	10	10.3	9
t	mean \pm SD	19.4 \pm 9.6	16.2 \pm 8.7	17.2 \pm 9.0	16.4 \pm 9.4
	median	18.5	15	16	14
	first quartile	15	8.5	9.8	10
	third quartile	25.5	22	24.3	22.5
dic	mean \pm SD	1.7 \pm 1.1	0.9 \pm 1.2	1.1 \pm 1.2	0.9 \pm 1.0
	median	2	0	1	1
	first quartile	1.25	0	0	0
	third quartile	2	1	2	1.5
ace	mean \pm SD	5.3 \pm 3.8	2.9 \pm 2.5	3.7 \pm 3.1	3.2 \pm 2.7
	median	4	2	3	3
	first quartile	3	1	1	1
	third quartile	5.75	4	5.3	4
complex	mean \pm SD	1.4 \pm 1.1	1.6 \pm 1.6	1.6 \pm 1.5	1.31 \pm 1.0
	median	1	1	1	1
	first quartile	1	0	0.8	1
	third quartile	2	2.75	2	2
F _C /100	mean \pm SD	2.65 \pm 1.32	2.27 \pm 1.18	2.40 \pm 1.23	2.28 \pm 1.22
	median	2.33	2.10	2.33	1.84
	first quartile	1.90	1.38	1.51	1.43
	third quartile	3.70	3.08	3.15	3.00

*number of current/former smokers in parenthesis

Abbreviations: Ab.c., aberrant cells/1000 cells; rcp, reciprocal translocations/1000 cells; t, total number of translocations/1000 cells; dic, dicentric chromosomes/1000 cells; ace, acentric chromosomes/1000 cells; complex, cells with complex rearrangement (three or more breaks in two or more chromosomes)/1000 cells; F_C/100, genomic frequency of stable translocations/100 cells.

32 breast cancer patients by FISH. Stable aberrations essentially contributed to the increased number of aberrant cells detected in their study.

The advantage of FISH over conventional analysis is the ability to detect stable aberrations. However, FISH is also able to detect unstable aberrations represented by dicentric chromosomes and acentric fragments. Though the results are limited to the fraction of the genome painted by FISH, more cells are commonly evaluated by this method than by a conventional one. Fluorescence staining is less appropriate for visualization of chromosome breaks and, therefore, these aberrations were not evaluated by FISH.

The case-control design of this study is not appropriate to discern if chromosomal aberrations are a biomarker for cancer risk or are the consequence of disease progression. However, large cohort studies substantiate the use of chromosome aberrations as an early predictive biomarker. Chromosome aberration frequency appears to be particularly associated with gastrointestinal cancer, mainly for stomach cancer [2, 3, 24]. For breast cancer, the results were only suggestive of an association. As these prospective studies evaluated unstable aberrations using conventionally stained chromosomes, the sensitivity of the biomarker can be compromised. Stable aberrations, which tend to accumulate

Table 5. Odds ratios of cytogenetic parameters for smokers in comparison with nonsmokers (adjusted for age).

	Controls				Gastrointestinal cancer				Breast cancer				All cancers			
	aOR	95% CI		p-value	aOR	95% CI		p-value	aOR	95% CI		p-value	aOR	95% CI		p-value
		lower	upper			lower	upper			lower	upper			lower	upper	
Ab.c.	1.27	1.09	1.48	0.002	1.04	0.90	1.19	0.588	1.03	0.93	1.14	0.591	1.05	0.97	1.13	0.251
rcp	1.24	1.04	1.48	0.014	1.05	0.87	1.26	0.636	1.06	0.94	1.20	0.325	1.06	0.96	1.17	0.260
t	1.10	1.02	1.19	0.012	1.03	0.95	1.12	0.513	1.01	0.95	1.07	0.752	0.92	0.78	1.08	0.315
dic	1.20	0.73	1.98	0.468	0.66	0.35	1.23	0.190	0.87	0.58	1.30	0.494	0.90	0.67	1.22	0.505
ace	1.26	1.02	1.55	0.035	0.99	0.80	1.23	0.918	1.05	0.89	1.25	0.560	1.07	0.94	1.21	0.289
complex	2.01	1.16	3.49	0.013	1.26	0.61	2.62	0.535	1.15	0.82	1.61	0.429	1.11	0.83	1.49	0.487
F _C /100	2.29	1.27	4.13	0.006	1.22	0.65	2.28	0.533	1.13	0.76	1.67	0.558	0.33	1.17	0.85	1.624

Abbreviations: Ab.c., aberrant cells/1000 cells; rcp, reciprocal translocations/1000 cells; t, total number of translocations/1000 cells; dic, dicentric chromosomes/1000 cells; ace, acentric chromosomes/1000 cells; complex, cells with complex rearrangement (three or more breaks in two or more chromosomes)/1000 cells; F_C/100, genomic frequency of stable translocations/100 cells; aOR, adjusted odds ratio; CI, confidence interval; significant values are in bold (p<0.05)

in the organism, may more reliably reflect increased susceptibility to chromosomal damage. Thus, translocations detected by chromosome painting may provide a cancer risk estimate superior to the traditional chromosome aberration analysis. In our study, we demonstrated that increased levels of stable translocations are associated with breast cancer. No association was revealed between stable cytogenetic parameters and TNM stages in any patient group. However, a limited number of patients, especially in the group of gastrointestinal cancer and prevailing lower stages in breast cancer patients, did not enable us to reach an undisputed conclusion on this topic. As regards unstable aberrations, a lower level of dicentric chromosomes in advanced TNM stages in our gastrointestinal cancer patients could be influenced by different exposure to X-rays between TNM groups during the diagnostic examination.

Tobacco smoke is one of the most important carcinogens and thus smoking is an established risk factor associated with many cancers, including gastrointestinal cancer [22]. It has been shown that cigarette smoking increases chromosome aberrations [25–28]. This is in agreement with our observation in healthy controls where chronic exposure to tobacco smoke increased the frequencies of stable and unstable chromosome aberrations except the dicentric chromosomes. However, this smoking related difference was not observed in groups of cancer patients. Furthermore, an association between chromosome damage and cancer observed in nonsmokers was unpronounced in smokers (with the exception of dicentric chromosomes in gastrointestinal patients). A similar effect of exposure to tobacco smoke and environmental pollution on chromosome aberrations was detected in other studies [8, 15]. It has been postulated that chronic exposure to environmental stressors can lead to adaptation of organisms via epigenetic mechanisms, which helps to reduce additional DNA damage [29]. It is well documented that genome methylation is strongly affected by smoking habits. Some sites remain differentially methylated for several decades after smoking cessation [30–33]. Thus, adaptation to chronic exposure of tobacco smoke in former and current

smokers probably helped to reduce the level of additional DNA damage in our cancer patients.

Different types of chromosome damage were observed in two groups of untreated cancer patients using FISH method with whole chromosome painting probes. Increased frequency of unstable aberrations detected in gastrointestinal cancer patients can be at least partly ascribed to acute exposure to diagnostic X-rays. In contrast, stable rearrangements that can persist in the body and cells with complex rearrangements contributed to chromosome damage in breast cancer patients.

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