ELSEVIER

Cancer Genetics and Cytogenetics 147 (2003) 62–67

Cancer Genetics and Cytogenetics

Comparative cytogenetic and DNA flow cytometric analysis of 242 primary breast carcinomas

Adewale Adeyinka[a], Bo Baldetorp[b], Fredrik Mertens[a,*], Håkan Olsson[b], Oskar Johannsson[b], Sverre Heim[d], Nikos Pandis[a,c]

[a]*Department of Clinical Genetics, University Hospital, Lund SE-221 85, Sweden*
[b]*Department of Oncology, University Hospital, Lund SE-221 85, Sweden*
[c]*Department of Genetics, Saint Savas Hospital, 171 Alexandras Avenue, 11522 Athens, Greece*
[d]*Department of Cancer Genetics, The Norwegian Radium Hospital, N-0310 Oslo, Norway*

Received 16 January 2003; received in revised form 14 April 2003; accepted 15 April 2003

Abstract The cytogenetic and DNA flow cytometric findings in 242 breast carcinomas were compared. The combined use of both techniques improved the detection of abnormal cell populations from 65% by cytogenetic analysis alone and 59% by DNA flow cytometric analysis alone to 84%. Informative and comparable cytogenetic and flow cytometric data were obtained for 155 tumors. Among these 155 tumors, there was good concordance (64%) between the estimates of genomic changes by the two methods. Most discrepancies were among the DNA-diploid cases, where cytogenetic analysis detected small genomic changes. There were, however, also some exceptions in which large genomic changes detected by one method were missed by the other. Of the specific breast cancer–associated cytogenetic aberrations subjected to separate correlation analysis, polysomy for chromosome 20 was significantly associated with a high S-phase fraction, whereas loss of the long arm of chromosome 16 and/or the presence of a der(1;16) were significantly associated with a low S-phase fraction. Our data show that cytogenetic and DNA flow cytometric analyses of breast carcinomas give largely comparable results, and that combining data from both methods significantly improves the information obtained by either technique used alone on the genetic abnormalities in these tumors.

1. Introduction

Cytogenetic and DNA flow cytometric analyses are both used to characterize the genomic changes of tumor cells. Both methods require the physical disruption of tissues, making it impossible to ascertain the morphology of the analyzed cells. Cytogenetic (CG) analysis is dependent on the harvesting of metaphase cells from short-term cultures of tumors or from cells already undergoing mitosis in vivo (direct harvest). The data generated may depend on the type of technique used (short-term culture or direct harvest), as well as on the fraction of tumor cells surviving and proliferating in culture [1,2]. On the other hand, DNA flow cytometric (FCM) analysis is rapid, allows the examination of a large number of cells, and can be performed on fresh, frozen, or paraffin-embedded tissue. However, there are uncertainties about the populations of cells represented by diploid/peridiploid DNA histograms [3], and DNA diploidy does not exclude the possibility of karyotypic changes in a tumor. For example, in tumors with karyotypic changes leading to minimal or no net loss or gain of genetic material, as well as in cases where the fraction of nondiploid tumor cells is small, FCM will not be able to detect the aberrant clones.

A few studies have combined and compared CG and FCM analyses in breast cancer [4–6] and in other types of malignant tumors [7–11]. In the present study, we compared the findings obtained from cytogenetic analysis of short-term cultures of tumor cells with DNA flow cytometric findings in 242 primary breast tumors.

2. Materials and methods

2.1. Patients

Samples were obtained from a series of 242 breast cancer patients who underwent surgery in Lund, Sweden from

* Corresponding author. Tel.: +46-46-173387; fax: +46-46-131061.
E-mail address: fredrik.mertens@klingen.lu.se (F. Mertens).

0165-4608/03/$ – see front matter
doi: 10.1016/S0165-4608(03)00190-0

December 1990 to December 1992 and from February 1995 to June 1997. All samples were from primary tumors and the patients were untreated at the time of sampling.

2.2. Cytogenetic study

Tissue sections adjacent to those used for histopathological examination were processed for short-term culture and cytogenetic analysis as described previously [12]. Briefly, tumor samples were mechanically and enzymatically disaggregated. The resulting cells and cell clumps were plated out in vitrogen-coated or Primaria flasks. Harvesting of metaphase cells (after 4–8 days in culture) involved exposure to Colcemid for 4–6 hours, trypsinisation to dislodge cells from the flask, hypotonic shock in 0.05 mol/L KCl, and fixation in methanolacetic acid (3:1). G-banding of chromosomes was obtained with Wright's stain. The clonality criteria and description of karyotypes followed the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN 1995) [13]. A chromosome index (CI) was calculated by dividing the modal chromosome numbers by 46, irrespective of the size of the chromosome(s) gained or lost. The karyotypes of the tumors have been reported and summarized [14,15].

2.3. DNA flow cytometry

Freshly frozen tumor samples (100 mg) were prepared for DNA flow cytometric analysis and were analyzed within 1 hour in an Ortho-Cytoron Absolute (Ortho Diagnostic Systems, Raritan, NJ) [16,17]. The DNA content per nucleus was quantitated from 20,000 nuclei per sample, and for each sample was presented as a DNA frequency distribution reflecting the different phases of the cell cycle (i.e., DNA histogram). Calculation of the DNA index (DI) was done after zero point adjustment of the DNA histogram using the modal DNA content values from the trout and chicken red blood cells included in the preparation of each sample. The mean channel numbers of all G0/G1 peaks in the histogram were then used for calculating the DNA indices, with the DNA-diploid G0/G1 peak as reference standard. Thus, a DNA-diploid stemline was defined as having a DNA index of 1.00. Furthermore, to be considered as a stemline, there should be a corresponding G2/M peak. The S-phase fraction (a measure of the proportion of cells in the DNA synthesis phase of the cell cycle) was calculated according to the planimetric method [18,19].

2.4. Comparison of cytogenetic and DNA flow cytometric findings

Cytogenetic and flow cytometric analyses were considered concordant when both techniques detected genetic abnormalities or failed to detect any genetic abnormalities in the same sample. In samples where both techniques detected genetic abnormalities, the abnormal clones were classified as "matching clones" if the difference between the CI and DI did not exceed 0.16.

2.5. Statistics

For statistical analysis, the Kruskal-Wallis test, chi square test, Normal test, Mann-Whitney U test, and Spearman's coefficient of correlation were used whenever appropriate. For all tests, statistical significance was considered to be at the $P < 0.05$ level.

3. Results

Table 1 summarizes the CG and FCM outcome for all 242 tumors investigated; 21 tumors had a normal karyotype, 159 had abnormal karyotypes, and 62 failed to grow in culture. Sixty-eight tumors were diploid, 142 were nondiploid, and 32 were unevaluable by FCM. Table 2 shows the FCM results and the chromosome indices of the 139 cases with abnormal karyotypes and informative FCM data. There was no significant difference between the medians of the S-phase fraction (SPF) of tumors with a normal karyotype, tumors that failed to grow in culture, and tumors with abnormal karyotypes ($P = 0.1354$, Kruskal-Wallis test).

Both techniques combined increased the fraction of tumors in which genetic abnormalities were detected from 65% and 59% by CG and FCM analysis, respectively, to 84%. CG and FCM data were available for comparison in 155 tumors (Table 1). Among these, there was a 64% concordance between the two techniques. Both methods detected matching abnormal clones with a good positive correlation between the CI and DI ($r_s = 0.90$, $P < 0.0001$) in 31 of the 97 cases that had abnormal karyotypes and nondiploid DNA indices. The most frequent discrepancies were seen among the DNA-diploid cases ($n = 68$), in which CG analysis detected aberrations in 62% of the cases ($Z = 3.61$, $P < 0.001$, Normal test). CG analysis, although more powerful than FCM in detecting small genomic changes, on the other hand, had a significantly higher failure

Table 1
Outcome of cytogenetic and DNA flow cytometric analyses of 242 breast tumors

Cytogenetics	DNA-diploid	DNA-nondiploid	Noninformative	Total
Normal karyotype	**2 (1%)**	**14 (6%)**	5 (2%)	21 (9%)
Abnormal karyotype	**42 (17%)**	**97 (40%)**	20 (8%)	159 (65%)
Failure in culture	24 (10%)	31 (13%)	7 (3%)	62 (26%)
Total	68 (28%)	142 (59%)	32 (13%)	242 (100%)

Boldface indicates cases that were evaluable with both techniques.

Table 2
CI, DI, and SPF of 139 tumors with abnormal karyotypes and informative FCM data

Serial no.	CI	DI	SPF (%)	Chromosome aberration[a]
1	0.91	1.00	13	1
2	0.93	1.00	6.6	3
3	0.96; 1.02	1.00	3	1;4;6
4	0.96; 1.00	1.00	5.6	
5	0.98; 1.00; 1.02	1.00	3.3	2; 6
6	0.98; 1.00	1.00	1.8	1; 4
7	0.98; 1.00	1.00	11	
8	0.98; 1.02	1.00	3.3	1; 4; 7
9	0.98	1.00	3.8	
10	1.00; 1.02; 2.04	1.00	4.3	
11	1.00; 1.02	1.00	3.4	1
12	1.00; 1.02	1.00	1.9	5
13	1.00; 1.04; 1.35	1.00	5.5	2; 3; 4; 5
14	1.00; 2.00	1.00	4.2	
15	1.00; 2.00	1.00	7.2	2
16	1.00; 2.00	1.00	4.3	1
17	1.00	1.00	5.7	
18	1.00	1.00	5.4	
19	1.00	1.00	3.7	
20	1.00	1.00	4.4	
21	1.00	1.00	6.2	
22	1.00	1.00	3	2
23	1.00	1.00	4	1; 4
24	1.00	1.00	6.1	
25	1.00	1.00	4.5	
26	1.00	1.00	1.2	
27	1.00	1.00	3.5	3
28	1.00	1.00	5.2	
29	1.00	1.00	5.4	
30	1.00	1.00	1.7	1; 4
31	1.02; 1.3	1.00	4.6	1; 4
32	1.02; 2.04	1.00	2.2	1; 3; 4
33	1.02	1.00	1.8	6
34	1.02	1.00	4.5	1
35	1.02	1.00	8.6	1
36	1.02	1.00	7	
37	1.02	1.00	5.3	
38	1.02	1.00	1.3	5
39	1.02	1.00	3.6	6
40	1.83; 3.65	1.00	2.2	1; 2
41	1.87; 3.7	1.00	4.3	
42	0.98; 1.17	1.00	13	6
43[b]	0.72; 1.5	0.86; 1.00	13	3; 6
44[b]	0.74; 1.48; 1.39; 1.02	0.80; 1.00	9.2	1; 2; 4
45[b]	0.78	0.89; 1.78; 1,00	NA	2; 3
46[b]	0.83; 1.74; 1.83	0.89; 1.00	3.6	1; 4
47	0.93	1.76; 1.00	5.9	4
48	0.96	1.53; 1.00	6.5	
49	0.96; 1.00	1.79; 1.00	9.3	
50	0.96; 1.02	1.84; 1.00	14	
51	0.96	1.89; 1.00	8.3	3
52	0.98; 1.00; 1.09	1.22; 2.44; 1.00	NA	
53	0.98; 1.00	1.59; 1.00	15	
54	0.98; 1.04	1.63; 1.00	NA	7
55[b]	1.00	0.91; 1.00	8.7	2
56[b]	1.00	0.96; 1.00	5.3	2
57[b]	1.00	1.06; 1.00	2.6	
58[b]	1.00	1.07; 1.00	3	1; 4
59[b]	1.00; 0.98	1.10; 1.16; 1.00	4.3	2
60[b]	1.00	1.12; 1.00	3	
61[b]	1.00	1.14; 1.00	4.9	
62[b]	1.00	1.14; 1.00	3	
63[b]	1.00; 1.04	1.15; 1.00	2.3	1
64[b]	1.00; 1.57	1.15; 1.00	8.7	2; 6; 5
65[b]	1.00	1.21; 1.09; 1.00	5.7	
66	1.00	1.20; 1.00	4.5	3
67	1.00; 1.02	1.28; 1.00	8.1	7
68[b]	1.00; 1.37	1.52; 1.00	NA	1; 2; 3
69	1.00	1.51; 1.00	11	
70	1.00; 2.00	1.53; 1.00	11	2
71	1.00; 1.02	1.55; 1.00	12	1; 5
72	1.00	1.55; 1.00	9.4	1; 4
73	1.00	1.57; 1.00	6.5	2
74	1.00; 0.93	1.57; 1.00	12	2
75	1.00	1.61; 1.00	NA	
76[b]	1.00; 1.67	1.62; 1.00	NA	1; 5; 6; 7
77	1.00	1.63; 1.00	15	
78	1.00	1.72; 1.00	19	
79	1.00	1.67; 1.00	12	
80	1.00	1.71; 0.86; 1.00	9.2	
81	1.00; 1.13; 0.96; 0.98	1.75; 1.00	NA	1; 6
82	1.00	1.77; 1.00	NA	3
83	1.00; 2.00	1.78; 1.00	NA	2
84	1.00; 1.14	1.77; 1.95; 1.00	11	1; 4; 5; 6
85	1.00	1.87; 1.00	9.3	
86	1.00; 1.02	1.88; 1.00	NA	5
87	1.00	1.85; 1.00	6.3	
88	1.00; 0.93	1.84; 1.00	16	
89	1.00	1.88; 1.00	11	3
90[b]	1.00; 1.93	1.90; 1.00	6.1	4
91	1.00; 1.02	1.94; 1.00	10	6
92[b]	1.00; 2.00; 2.05	1.96; 1.00	4.4	1; 2; 3; 4; 5
93	1.00; 1.04	1.95; 1.00	7.1	3; 6; 7
94	1.00	1.99; 1.00	16	1
95	1.00	1.93; 1.00	NA	2
96[b]	1.00	1.98; 1.16; 1.00	8.8	1; 4
97[b]	1.00; 2.00	2.02; 1.87; 1.00	2.9	1; 4
98	1.00; 1.07; 1.65	2.10; 1.42; 1.00	NA	1; 4; 5; 7
99	1.00	2.18; 1.00	NA	
100	1.00; 1.02	2.14; 1.00	15	
101	1.00; 1.17	2.17; 1.00	15	1; 2; 4; 7
102	1.00	2.12; 1.00	13	1; 2
103	1.00; 0.98	2.52; 1.00	21	
104[b]	1.02	1.07; 1.00	13	5; 6
105[b]	1.02	1.08; 1.00	6	1
106[b]	1.02	1.07; 1.00	7.4	
107	1.02	1.20; 1.00	8	1; 4
108	1.02	1.33; 1.00	13	5
109	1.02	1.36; 1.00	NA	5
110[b]	1.02; 1.43	1.44; 1.00	NA	1; 7
111	1.02; 1.04	1.60; 1.00	11	6
112	1.02; 1.00	1.76; 1.00	7.3	
113	1.02	1.68; 1.00	13	6
114	1.02; 1.00	1.77; 1.00	11	1; 3; 4; 5
115	1.02; 1.28; 2.33	1.77; 1.00	NA	1; 2
116	1.02; 1.07; 1.57	1.77; 1.00	14	1; 6; 7
117	1.02; 1.00	1.80; 1.00	11	5
118	1.02	1.82; 1.56; 1.00	13	5
119	1.02	1.83; 1.00	7.5	5
120	1.02; 1.00	1.86; 1.00	7.5	5

(*continued*)

Table 2
(Continued)

Serial no.	CI	DI	SPF (%)	Chromosome aberration[a]
121[b]	1.02; 1.04; 1.07; 1.96	1.91; 1.00	9.9	5; 7
122	1.02	1.97; 1.00	NA	
123	1.02; 0.98	3.07; 1.00	14	6
124	1.04	2.02; 1.00	9.8	
125	1.09	1.89; 1.00	NA	5
126	1.22	2.00; 1.00	7.6	6
127	1.37; 1.00	1.12; 1.00	7.5	3
128[b]	1.43; 1.00	1.50; 1.00	22	5; 7
129[b]	1.48	1.53; 1.00	7.8	1; 2; 7
130	1.48	1.85; 1.00	NA	1; 3
131	1.52	1.72; 1.00	14	1; 7
132[b]	1.54	1.69; 1.00	NA	3
133	1.57	1.75; 1.00	10	
134	1.63	1.09; 1.00	4.3	1; 5; 6
135[b]	1.65	1.73; 1.00	11	1
136	1.65	1.94; 1.00	9.8	3; 6
137	1.84	2.34; 1.00	8.8	3; 7
138	2.08	2.29; 1.00	9.7	2; 7
139[b]	2.26; 4.52	2.25; 1.00	3.5	

Abbreviation: NA, not available.

[a] Characteristic chromosome aberrations of breast cancer found at cytogenetic analysis. 1, gain of 1q or i(1q); 2, loss of 3p or del(3p); 3, loss of 6q or del 6(q); 4, loss of 16q or der(1;16); 5, +7; 6 = +18; 7 = +20.

[b] Tumors with matching abnormal clones detected by cytogenetic and flow cytometric analyses.

rate ($Z = 3.24$, $P < 0.002$, Normal test; Table 1). Among all karyotypically abnormal tumors, the mean DI was higher than the mean CI (Table 3).

Regarding the specific cytogenetic features, there was no significant difference in the distribution of cytogenetic polyclonality among the DNA-diploid and DNA-nondiploid tumors ($P = 0.9410$, chi square test, Table 4). Tumors with polysomy for chromosomes 7, 18, or 20 showed higher SPF values (median: 9.7%–9.9%) than tumors without these polysomies (median: 6.5%–6.55%). These differences were, however, statistically significant only for +20 ($P = 0.0277$, Mann-Whitney U test). Loss of the long arm of chromosome 16, including karyotypes with the der(1;16) translocation that simultaneously leads to loss of 16q and gain of 1q, was significantly associated with a low SPF ($P = 0.0159$, Mann-Whitney U test; Table 5).

4. Discussion

The cytogenetic and DNA flow cytometric findings for 242 human primary breast carcinoma samples were compared. For those tumors where both cytogenetic and DNA flow cytometric data were available for comparison, there was a reasonably good concordance rate of 64% in detecting clones with genetic abnormalities. Previous studies combining data from CG and FCM for the analysis of aneuploidy in solid tumors have reported a wide range of concordances: 37%–73% for bladder cancer [20,21], 43% for prostate cancer [9], 30% for various solid tumors [22], 69% for pediatric solid tumors [10], 54% for bone and soft tissue tumors [11], and 54% for breast carcinomas [6]. As in the present study, most of the discrepancies occurred in cases that exhibited near diploidy by cytogenetics and diploid-DNA histograms, a finding that can be accounted for by the inherent inability of FCM analysis to detect balanced rearrangements or gains or losses amounting to less than 4% of the normal DNA content [23]. On average, and especially among the DNA-nondiploid tumors, the DI was slightly higher than the CI (Table 3). Similar findings have been reported in other studies, implying that abnormal chromosomes are often larger than normal chromosomes [4–7,11]. The size of marker chromosomes was not taken into consideration in the calculation of the CI.

Technical difficulties and failure of cells to grow in culture limit the possibility of obtaining interpretable cytogenetic data from short-term cultures of solid tumors. Similarly, because of inadequate sampling, informative DNA histograms cannot be obtained from all tumor samples. Also, in the presence of debris or multiple or overlapping aneuploid populations of cells, SPF cannot be estimated from all DNA histograms [24]. It might have been expected that a high SPF, which indicates a high proliferative activity in vivo, would have been reflected in better proliferation of tumor cells in culture. This was not the case in the present study, as there was no difference in the median SPF values of tumors that failed to grow in culture, those with a normal karyotype, and those that had abnormal karyotypes. A plausible explanation for this would be that tumor sampling played an important role in determining the cytogenetic outcome—failure in culture, detection of a normal karyotype, or detection of chromosome abnormalities.

Multiple cytogenetic clones were detected in 72 (52%) of the karyotypically aberrant tumors (Table 4). Multiple abnormal cell populations were also detected, albeit to a lesser extent, by FCM analysis. This heterogeneity could partly explain the discrepancies between the two types of analysis. Although samples for CG and FCM analyses were from adjacent areas of the tumors, the clones present in the sample used for one analysis may not necessarily have

Table 3
Comparison between DI and CI of 139 karyotypically aberrant tumors

FCM	Cytogenetics	No. of tumors	DI		CI	
			Mean	SD	Mean	SD
Diploid	CI = 1	14	1.00	± 0.00	1	± 0.00
	CI> or <1	28	1.00	± 0.00	1.40	± 0.36
Nondiploid	CI = 1	26	1.50	± 0.40	1	± 0.00
	CI> or <1	71	1.68	± 0.41	1.29	± 0.91

Table 4
Distribution of cytogenetic polyclonality in DNA-diploid and DNA nondiploid tumors

Category	DNA diploid	DNA nondiploid	Total
Cytogenetic polyclonality	50	22	72
Cytogenetic monoclonality	47	20	67
Total	97	42	139

Table 5
Mann-Whitney *U* test results for SPF in relation to specific chromosome aberrations

	Tumors with aberration		Tumors without aberration		
Chromosome aberration	n[a]	Median SPF [range] (%)	n[b]	Median SPF [range] (%)	*P*
Gain of 1q or i(1q)	34	5.30 [1.7–16]	85	7.30 [1.2–22]	0.2178
Loss of 3p or del(3p)	18	7.50 [2.2–15]	101	7.10 [1.2–22]	0.9852
Loss of 6q or del(6q)	13	7.10 [2.2–13]	106	7.25 [1.2–22]	0.7559
Loss of 16q or der(1;16)	21	4.60 [1.7–15]	98	7.50 [1.2–22]	0.0159*
+7	17	9.90 [1.3–22]	102	6.55 [1.2–21]	0.1789
+18	17	9.80 [1.8–14]	102	6.55 [1.2–22]	0.2877
+20	11	9.70 [3.3–22]	108	6.50 [1.2–21]	0.0277*

[a] Number of tumors with a particular chromosome abberation and for which SPF values were available.
[b] Number of tumors without a particular chromosome abberation and for which SPF values were available.
* Statistically significant.

been represented in the sample used for the other. Multiple sampling of different areas of a tumor is known to yield different clones upon cytogenetic analysis [25]. In addition, the cytogenetic analysis was carried out on cultured tumor cells with the further possibility of selection in vitro [1,2]. As a result, sampling of multiple areas of a tumor for both CG and FCM analyses may give a more representative picture of the whole spectrum and distribution of clones.

Measurement of the SPF is said to have clinical implications for patients with breast cancer; higher SPF is generally associated with high tumor grade, positive axillary lymph nodes, absence of steroid receptors, and shorter disease-free and overall survival [26]. We therefore compared the characteristic chromosome aberrations of breast carcinomas [27] with the SPF in the tumors analyzed. Tumors with polysomy for chromosome 7, 18, or 20 showed increased SPF values, although this association was only statistically significant for +20. Loss of the long arm of chromosome 16, on the other hand, was significantly associated with a low SPF (Table 5). Other studies using comparative genomic hybridization and fluorescence in situ hybridization have shown a significant association between amplifications of 20q and a high histological grade, DNA aneuploidy, a high SPF, and an early relapse of breast carcinoma [28,29]. Furthermore, conventional cytogenetic, molecular cytogenetic, and molecular genetic studies have indicated that loss of the long arm of chromosome 16, alone or in combination with the gain of 1q that results from a der(1;16) translocation, as well as allelic loss of 16q23.2~q24.2, are possible indicators of good prognosis for patients with breast cancer [15,30,31]. Although net gain of 1q can be achieved in many different ways, the seemingly low median SPF values of tumors with gain of 1q (Table 5) may possibly be attributed to loss of 16q because such deletions were present in 18 of the 34 tumors with gain of 1q. These 18 tumors had a median SPF of 4.2% (range: 1.7%–15%), whereas the remaining 16 tumors with +1q and no concomitant loss of 16q had a median SPF of 8.2% (range: 2.2%–16%). Hence, the present study provides further support for the association between loss of 16q material and a less aggressive tumor phenotype.

Acknowledgments

This work was supported by grants from the Swedish Cancer Society, the Swedish Society for Medical Research, the Inga Britt and Arne Lundberg Foundation, the John and Augusta Persson Foundation, the Medical Faculty of Lund University, and the King Gustav V Jubilee Foundation.

References

[1] Pandis N, Bardi G, Heim S. Interrelationship between methodological choices and conceptual models in solid tumor cytogenetics. Cancer Genet Cytogenet 1994;76:77–84.
[2] Steinarsdóttir M, Pétursdóttir I, Snorradóttir S, Eyfjörd JE, Ögmundsdóttir HM. Cytogenetic studies of breast carcinomas: different karyotypic profiles detected by direct harvesting and short-term culture. Genes Chromosomes Cancer 1995;13:239–48.
[3] Alvegård TA, Berg NO, Baldetorp B, Fernö M, Killander D, Ranstam J, Rydholm A, Åkerman M. Cellular DNA content and prognosis of high-grade soft tissue sarcoma. The Scandinavian Sarcoma Group experience. J Clin Oncol 1990;8:538–47.
[4] Remvikos Y, Gerbault-Seurreau M, Vielh P, Zafrani B, Magdelénat H, Dutrillaux B. Relevance of DNA ploidy as a measure of genetic deviation: a comparison of flow cytometry and cytogenetics in 25 cases of human breast cancer. Cytometry 1988;9:612–8.
[5] López-Ginés C, Callaghan RC, Ruiz A, Gil R, Pellín A, Calderón J, Vazquez C, Llombart-Bosch A. Cytogenetics, flow cytometry, cytophotometry and morphometry of 22 cases of primary breast carcinoma. A comparative study. Virchows Arch B Cell Pathol 1991;61:133–40.
[6] Steinarsdóttir M, Jónasson JG, Pétursdóttir I, Sigurdsson H, Ögmundsdóttir HM. A comparison of cytogenetic studies and flow cytometry in breast carcinomas. Cytometry 1997;28:323–8.
[7] Petersen SE, Friedrich U. A comparison between flow cytometric ploidy investigation and chromosome analysis of 32 human colorectal tumors. Cytometry 1986;7:307–12.
[8] Tribukait B, Granberg-Öhman I, Wijkström H. Flow cytometric DNA and cytogenetic studies in human tumors: a comparison and discussion of the differences in modal values obtained by the two methods. Cytometry 1986;7:194–9.
[9] Bratt O, Anderson H, Bank-Jesen E, Baldetorp B, Lundgren R. Metaphase cytogenetics and DNA flow cytometry with analysis of S-phase fraction in prostate cancer: influence in prognosis. Urology 1996;2:218–24.
[10] Dressler LG, Duncan MH, Varsa EE, Macconnell TS. DNA content measurement can be obtained using archival material for DNA flow

cytometry. A comparison with cytogenetic analysis in 56 pediatric solid tumors. Cancer 1993;72:2033–41.

[11] Mandahl N, Baldetorp B, Fernö M, Åkerman M, Rydholm A, Heim S, Willén H, Killander D, Mitelman F. Comparative cytogenetic and DNA flow cytometry analysis of 150 bone and soft-tissue tumors. Int J Cancer 1993;53:358–64.

[12] Pandis N, Heim S, Bardi G, Limon J, Mandahl N, Mitelman F. Improved technique for short-term culture and cytogenetic analysis of human breast cancer. Genes Chromosomes Cancer 1992;5:14–20.

[13] Mitelman F, editor. An international system for human cytogenetic nomenclature. Basel: S. Karger, 1995.

[14] Pandis N, Idvall I, Bardi G, Jin Y, Gorunova L, Mertens F, Olsson H, Ingvar C, Beroukas K, Mitelman F, Heim S. Correlation between karyotypic pattern and clinicopathologic features in 125 breast cancer cases. Int J Cancer 1996;66:191–6.

[15] Adeyinka A, Mertens F, Idvall I, Bondesson L, Ingvar C, Mitelman F, Pandis N. Different patterns of chromosomal imbalances in metastasising and non-metastasising primary breast carcinomas. Int J Cancer 1999;84:370–5.

[16] Baldetorp B, Dalberg M, Holst U, Lindgren G. Statistical evaluation of cell kinetic data from DNA flow cytometry (FCM) by the EM algorithm. Cytometry 1989;10:695–705.

[17] Baldetorp B, Fernö M, Fallenius A, Fallenius-Vecchi G, Idvall I, Olsson H, Sigurdsson H, Åkerman A, Killander D. Image cytometric DNA analysis in human breast cancer analysis may add prognostic information in diploid cases with low S-phase fraction by flow cytometry. Cytometry 1992;13:577–85.

[18] Baisch H, Gohde W, Linden WA. Analysis of PCP-data to determine the fraction of cells in the various phases of cell cycle. Radiat Environ Biophys 1975;12:31–9.

[19] Baldetorp B, Bendahl PO, Fernö M, Alanen K, Delle U, Falkmer U, Hansson-Aggesjo B, Hockenstrom T, Lindgren A, Mossberg L, Nordling S, Sigurdsson H, Stål O, Visakorpi T. Reproducibility in DNA flow cytometric analysis of breast cancer: comparison of 12 laboratories' results for 67 sample homogenates. Cytometry 1995;22: 115–27.

[20] Coon JS, Schwartz D, Summers JL, Miller AW, Weistein RS. Flow cytometric analysis of deparaffinized nuclei in urinary bladder carcinoma. Comparison with cytogenetic analysis. Cancer 1986;57: 1595–601.

[21] Smeets AWGB, Pauwels RPE, Beck JLE, Beck JLM, Geraedts JPM, Debruyne FMJ, Laarakkers L, Feitz WFJ, Voous GP, Ramaaekers FCS. Tissue-specific markers in flow cytometry of urological cancers III. Comparing chromosomal and flow cytometry DNA analysis of bladder tumors. Int J Cancer 1987;39:304–10.

[22] Shackney SE, Burholt DR, Pollice AA, Smith CA, Pugh RP, Hartsock JR. Discrepancies between flow cytometric and cytogenetic studies in the detection of aneuploidy in human solid tumors. Cytometry 1990;11:94–104.

[23] Vindeløv LL, Christensen IJ, Jensen G, Nissen NI. Limits of detection of nuclear DNA abnormalities by flow cytometric DNA analysis. Results obtained by a set of methods for sample-storage, staining and internal standardization. Cytometry 1983;3:332–9.

[24] O'Reilly SM, Richards MA. Is DNA flow cytometry a useful investigation in breast cancer? Eur J Cancer 1992;28:504–7.

[25] Teixeira MR, Pandis N, Bardi G, Andersen JA, Mitelman F, Heim S. Clonal heterogeneity in breast cancer: karyotypic comparisons of multiple intra- and extra-tumorous samples from 3 patients. Int J Cancer 1995;63:63–8.

[26] Wenger RC, Clark GM. S-phase fraction and breast cancer- a decade of experience. Breast Cancer Res Treat 1998;51:255–65.

[27] Pandis N, Jin Y, Gorunova L, Petersson C, Bardi G, Idvall I, Johansson B, Ingvar C, Mandahl N, Mitelman F, Heim S. Chromosome analysis of 97 primary breast carcinomas: identification of eight karyotypic subgroups. Genes Chromosomes Cancer 1995;12:173–85.

[28] Isola J, Kallioniemi O-P, Chu LW, Fuqua SAW, Hilsenbec SG, Osborne KC, Waldman FM. Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. Am J Pathol 1995;147:905–11.

[29] Tanner MM, Tirkkonen M, Kallioniemi M, Holli K, Collins C, Kowbel D, Gray JW, Kallioniemi OP, Isola J. Amplification of chromosomal region 20q13 in invasive breast cancer: prognostic implications. Clin Cancer Res 1995;1:1455–61.

[30] Hansen LL, Yilmaz M, Overgaard J, Andersen J, Kruse TA. Allelic loss of 16q232–242 is an independent marker of good prognosis in primary breast cancer. Cancer Res 1998;58:2166–9.

[31] Tsuda H, Takarabe T, Fukutomi T, Hirohashi S. der(16)t(1;16)/der(1;16) in breast cancer detected by fluorescence in situ hybridization is an indicator of better prognosis. Genes Chromosomes Cancer 1999;24:72–7.