Array comparative genomic hybridisation (aCGH) analysis of premenopausal breast cancers from a nuclear fallout area and matched cases from Western New York

G Varma1, R Varma2, H Huang3, A Pryshchepava3, J Groth3, D Fleming3, NJ Nowak4, D McQuaid4, J Conroy4, M Mahoney5, K Moysich5, KL Falkner6 and J Geradts*,3,4

1Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA; 2Department of Biostatistics, Roswell Park Cancer Institute, Buffalo, NY, USA; 3Department of Pathology and Laboratory Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA; 4Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY, USA; 5Department of Cancer Prevention and Epidemiology, Roswell Park Cancer Institute, Buffalo, NY, USA; 6Department of Oral Biology, State University of New York, Buffalo, NY, USA

High-resolution array comparative genomic hybridisation (aCGH) analysis of DNA copy number aberrations (CNAs) was performed on breast carcinomas in premenopausal women from Western New York (WNY) and from Gomel, Belarus, an area exposed to fallout from the 1986 Chernobyl nuclear accident. Genomic DNA was isolated from 47 frozen tumour specimens from 42 patients and hybridised to arrays spotted with more than 3000 BAC clones. In all, 20 samples were from WNY and 27 were from Belarus. In total, 34 samples were primary tumours and 13 were lymph node metastases, including five matched pairs from Gomel. The average number of total CNAs per sample was 76 (range 35–134). We identified 152 CNAs (92 gains and 60 losses) occurring in more than 10% of the samples. The most common amplifications included gains at 8q13.2 (49%), at 1p21.1 (36%), and at 8q24.21 (36%). The most common deletions were at 1p36.22 (26%), at 17p13.2 (26%), and at 8p23.3 (23%). Belarusian tumours had more amplifications and fewer deletions than WNY breast cancers. HER2/neu negativity and younger age were also associated with a higher number of gains and fewer losses. In the five paired samples, we observed more discordant than concordant DNA changes. Unsupervised hierarchical cluster analysis revealed two distinct groups of tumours: one comprised predominately of Belarusian carcinomas and the other largely consisting of WNY cases. In total, 50 CNAs occurred significantly more commonly in one cohort vs the other, and these included some candidate signature amplifications in the breast cancers in women exposed to significant radiation. In conclusion, our high-density aCGH study has revealed a large number of genetic aberrations in individual premenopausal breast cancer specimens, some of which had not been reported before. We identified a distinct CNA profile for carcinomas from a nuclear fallout area, suggesting a possible molecular fingerprint of radiation-associated breast cancer.

The incidence of breast cancer in young women is lower than in the postmenopausal age group. However, carcinomas in these patients are generally more aggressive and associated with poorer prognosis. The aetiology of premenopausal breast cancer is not clear. In a minority of patients, tumours develop on the basis of germline mutations in the BRCA1 and BRCA2 genes. Environmental exposure variables have been extensively studied as a causative factor of human mammary neoplasia. There is significant evidence, derived from diverse populations, that ionising radiation can cause breast cancer. Some examples are patients who received therapeutic radiation to the chest early in life for Hodgkin’s disease (HD), patients who were treated with radiation for mastitis and other benign breast diseases, patients who received thymic irradiation, patients who underwent frequent fluoroscopies for pulmonary disease, and atomic bomb survivors (Behrens et al., 2000; Clemons et al., 2003; Gaffney et al., 2001; Land et al., 2003; Travis et al., 2003). Among these, the best-studied group are women who developed breast cancer after treatment for HD. It was reported that the risk of developing breast cancer was greatest if patients were treated under the age of 30, and hormonal stimulation of the irradiated breast tissue appeared to play an important role (Clemons et al., 2000; Travis et al., 2003). The risk clearly was dose dependent (Travis et al., 2003), and the median latency period was in the range of 15 years (Clemons et al., 2000). Similar observations held true for the Japanese atomic bomb survivors. Breast cancer risk was inversely related to age at exposure, there was a linear dose response, and the minimum latency period was 12 years (Land et al., 2003). Tumours arising in irradiated women
may be associated with reduced overall survival (Gaffney et al., 2001).

Researchers at Roswell Park Cancer Institute (RPCI), including some of the authors of this paper, have been actively involved in several epidemiologic studies on a more recent group of probands, namely individuals exposed to radiation released by the 1986 Chernobyl nuclear accident (Mahoney et al., 2004). Although the reactor was located in Ukraine, neighboring Belarus received about 70% of the radioactive fallout (Ermak et al., 2003). It is well documented that children exposed to that fallout have an increased cancer rate (Peterson et al., 1997). One of the most common radiation-induced malignancies is papillary carcinoma of the thyroid, and this tumour type was shown to have genetic abnormalities that differed from those in sporadic thyroid cancers (Tuttle and Becker, 2000; Ermak et al., 2003). There also was an increase in breast cancer incidence in parts of Belarus after the Chernobyl accident, especially in rural areas and in premenopausal women (Sasnouskaya and Okeanov, 2000).

In theory, breast carcinomas associated with ionising radiation should facilitate insight into the molecular pathogenesis of early-onset breast cancer, yet few such studies have been published. In one paper, post-HD breast carcinomas were found to have more microsatellite alterations compared to sporadic tumours (Behrens et al., 2000). In another group of post-HD breast cancer patients, there were no significant differences compared with sporadic cases with regard to BRCA1, BRCA2, oestrogen receptor (ER), PR, HER2 and p53 status (Gaffney et al., 2001). Several in vitro studies are of relevance. It was shown that human mammary epithelial cells (HMEC) in culture can be transformed by γ-irradiation (Wazer et al., 1994). Subsequent studies showed that radiation induced nonrandom chromosomal changes in HMEC (Durante et al., 1996; Yang et al., 1997). Finally, irradiation of nontransformed MCF-10F cells led to altered expression of 49 of 190 genes assayed (Roy et al., 2001).

Comparative genomic hybridisation (CGH) is one tool to investigate the molecular pathology of human tumours. Conventional CGH is based on competitive in situ hybridisation of normal metaphase spreads by two differentially labelled whole genomic DNAs, one derived from tumour tissue and the other from a normal reference. Regions of altered DNA copy number (losses and gains) in the tumour are quantitated as ratio changes along metaphase chromosomes. The resolution of this technique is in the range of 10–20 Mb. In this study, we have used array CGH to obtain better resolution to facilitate identification of novel candidate breast cancer genes. High-density bacterial artificial chromosome (BAC)-based arrays developed at RPCI were employed to screen for DNA copy number gains and losses in premenopausal breast cancers from two geographically distinct areas in an attempt to identify genetic changes that may be specific to early-onset tumours and/or radiation exposure.

**MATERIALS AND METHODS**

**Tissues**

In all, 55 frozen samples of breast cancers were obtained from premenopausal patients from Western New York (WNY) and Belarus. Samples from Belarus were collected and snap frozen between August 2002 and January 2003. The tumours were collected from women who resided in the Gomel area since April 1986. They were transported on dry ice to the United States and processed at RPCI. The WNY samples were obtained through the tissue procurement facility at RPCI. Patients with a strong family history of breast cancer and cases with known BRCA1/2 mutations were excluded. This study was approved by the RPCI Institutional Review Board. All samples were evaluated morphologically and only those with more than 50% tumour cellularity were included.

Preliminary experiments had shown that >30% tumour cellularity was sufficient. Duplicate assays and dye swapping experiments were performed for a subset of samples, showing very good reproducibility. Genomic DNA was extracted from all samples using TriZol (Invitrogen, Grand Island, NY, USA). In total, 47 samples met the quality criteria and were included in the final analysis. Patient characteristics are shown in Table 1. All Gomel patients were Caucasian, as were the great majority of RPCI patients (except for two African American women). The age of the patients ranged from 24 to 50 years. There were 34 primary tumours and 13 lymph node metastases. This cohort included five paired cases from Belarus.

Her2 protein expression assays

The immunohistochemical assay for Her2 expression in formalin-fixed, paraffin-embedded tumour sections followed the Herceptest protocol (Dako, Carpinteria, CA, USA), and the stains were scored from 0 and 1+ (negative) to 2+ and 3+ (positive), using recommended guidelines. For Western blotting, 30 μg of protein was used per sample (extraction from tissue utilized the TriZol protocol). Protein was resolved over 8% SDS–PAGE and transferred to a PVDF membrane. The blot was blocked in blocking buffer (5% nonfat dry milk, 10 mM Tris (pH 7.5), 10 mM NaCl, and 0.1% Tween-20) for 1 h at room temperature. The membrane was then incubated with the Herceptest antibody (Dako) at a dilution of 1:500 at 4°C overnight. This was followed by incubation with a goat anti-mouse horseradish peroxidase-conjugated secondary antibody (KPL, Gaithersburg, MD, USA) at a dilution of 1:3000 at room temperature for 1 h. Protein bands were visualised by the SuperSignal West Pico Chemiluminescent Substrate kit obtained from PIERCE (Rockford, IL, USA) and exposed with Kodak X-Ray film.

| Table 1 Patient characteristics | WNY | Belarus | P-value |
|--------------------------------|-----|---------|---------|
| Age (median, years)            | 41.5| 45.5    | 0.23    |
| 40−                           | 12  | 10      |         |
| 41−                           | 8   | 12      |         |
| Caucasian                      | 18  | 22      | 0.43    |
| African American               | 2   | 0       |         |
| Histology                      |     |         |         |
| Ductal                        | 17  | 20      |         |
| Lobular                       | 2   | 0       | 0.29    |
| Other/unknown                 | 1   | 2       |         |
| Grade                          |     |         |         |
| 1−2                           | 7   | 5       | 0.81    |
| 3                             | 13  | 14      |         |
| Unknown                        | 0   | 3       |         |
| Size                           |     |         |         |
| T1                            | 4   | 8       | 0.30    |
| T2–T4                         | 16  | 12      |         |
| Unknown                        | 0   | 2       |         |
| LN status                      |     |         |         |
| Negative                       | 6   | 5       | 0.85    |
| Positive                       | 14  | 17      |         |
| Stage                          |     |         |         |
| I                             | 2   | 4       |         |
| II                            | 11  | 12      | 0.71    |
| III                           | 7   | 6       |         |
| WNY = Western New York; LN = lymph node.
Array-based CGH (aCGH)

The CGH arrays were prepared in the Microarray Core Facility at RPCI according to established protocols (Snijders et al., 2001; Cowell and Nowak, 2003; Cowell et al., 2004a). A total of 3084 BAC clones were common to all arrays used in this series of experiments (average resolution ~1 Mb). The WNY and Belarusian samples were coded and then assayed concurrently and blindly. Genomic DNA (0.5 μg) was fluorescently labelled by random priming in a 100 μl reaction containing the DNA, random primers solution, appropriate buffers and Cy3- or Cy5-dCTP-labelled nucleotides. Labelling occurred with the addition of appropriate agents and incubation overnight at 37°C. Arrays were hybridised with appropriate solutions for 16h. Slides were washed, dried and immediately scanned using an Affymetrix 428 scanner (Cowell et al., 2004b). Image analysis was performed using the ImaGene version 4.1 software from BioDiscovery Inc. The reference was pooled normal male DNA. Output of the image analysis was processed by an in-house Perl program to calculate log-transformed and normalised mean ratios of test to reference fluorescence intensities. Any BAC that had less than two replicates flagged as good or a standard error greater than 0.15 was excluded. Map positions were identified by querying the human genome sequence (July 2003 Build) at http://genome.ucsc.edu. A sample output showing intensity ratios across the whole genome for an individual tumour is shown in Figure 1.

Data analysis

Copy number aberrations (CNAs) were determined at the clonal level for each individual coded sample in a blinded manner. The genome-wide mean and standard deviation of the log ratios were calculated for the autosomal chromosomes and the X chromosome separately. Thresholds for amplification and deletion were set at two standard deviations from the mean in both directions. Contiguous regions of amplification or deletion were identified by flagging clones on the array based on adjacent chromosomal locations. Recurrent amplifications and deletions were determined by the frequency of these events among all samples or within specific groups.

Unsupervised hierarchical clustering was performed using the TIGR Multi-experiment Viewer (MeV version 2.2) software (Saeed et al., 2003). Only clones that were included in at least 45 out of 47 samples were considered. A filtered set of 202 clones with a high variability across all samples (standard deviation/mean > 0.3) was used for the hierarchical clustering. Clusters were generated using the average linkage method with Pearson’s correlation coefficient as the similarity metric.

Association between groups was tested from contingency tables using the $\chi^2$ Pearson statistic. Resulting P-values are shown as appropriate.

RESULTS

In all, 47 samples that met all quality control criteria including tumour cellularity and patient age were included in the final analysis. Patient characteristics are shown in Table 1. The two groups from WNY and Gomel were well matched for ethnic background, age, extent of disease (tumour size, nodal involvement, stage), tumour histology, grade and ER status. The average number of CNAs in the breast carcinomas was 76 (37 gains and 39 losses). Tumours from WNY, older patients, and HER2-positive cancers had more deletions and fewer amplifications than tumours from Belarus, younger patients, and HER2-negative cancers, respectively (Table 2). Primary tumours and lymph node metastases, smaller and larger tumours,
ER+ and ER− cases, and carcinomas with and without associated nodal metastases had comparable frequencies of copy number gains and losses.

A representative diagram of genome-wide amplifications and deletions is shown in Figure 1. Most breast cancers displayed both distinct single BAC amplifications and deletions as well as broader areas of DNA gains and losses. A total of 152 CNAs occurred in more than 10% of samples, including 92 copy number gains and 60 losses. The majority of DNA gains (72%) were on the long arms of chromosomes 1 and 8. Most of them were gains of one or two copies, but a subset was higher level amplifications (three copies or more, Table 3). The most common amplifications were at 8q13.2 (49%), at 1p21.1 (36%), and at 8q24.21 (36%). Gains at loci containing the well-known breast oncogenes c-myc (at 8q24.21), HER2 (at 17q21.1), and cyclin D1 (at11q13.3) were also frequent (26, 19, and 13%, respectively) (Table 3). In an initial validation experiment, HER2 amplification detected by array CGH was correlated with protein expression using immunohistochemistry and Western blot analysis (Figure 2). Amplified tumours showed high levels of the Her2 oncoprotein, while nonamplified cases were negative. While the majority of chromosomal gains had previously been described, we identified seven novel recurrent gains (as indicated in Table 3), five of which were in areas on 8q that had not been reported to be common amplification sites in breast cancer.

The largest number of DNA losses was on chromosome 17. In most instances, one allele was lost, but possible homozygous deletions were also observed (Table 4). The most common deletions were at 1p36.22 (26%), at 17p13.2 (26%), and at 8p23.3 (23%). We identified nine novel recurrent losses, seven of which were on chromosome 22 (Table 4).

In the five paired samples, some genetic changes were common to the primary tumour and the lymph node metastasis. The number of shared changes varied markedly from case to case. However, all cases were characterised by a large number of discordant CNAs. Metastatic tumours consistently developed more gains than losses (Table 5).

Unsupervised hierarchical clustering using 202 discriminating BAC clones produced a dendrogram with two distinct arms: one predominantly composed of WNY carcinomas and the other mostly comprised of Belarusian samples (P < 0.001) (Figure 3). These two arms were not significantly different with regard to patient age, primary tumours vs metastases, tumour size, nodal status, stage, grade, ER, or HER2 status. A total of 50 BAC clones were differentially amplified or deleted in premenopausal breast cancers from WNY and Belarus, and 25 of these contained named genes (Table 6). Of particular interest were 10 BAC clones that were amplified selectively in Belarusian tumours. Moreover, three of these BACs were specifically deleted in WNY

Table 2  Distribution of CNAs

|                  | Average gains | Average losses | Average total CNAs |
|------------------|---------------|----------------|--------------------|
| WNY              | 32a           | 44             | 76                 |
| Belarus          | 42a           | 35             | 77                 |
| Age under median (≤43) | 43b          | 33b            | 76                 |
| Age over median (>43) | 33c          | 45c            | 78                 |
| Small tumours (T1) | 39            | 36             | 75                 |
| Large tumours (T2–T4) | 37            | 40             | 77                 |
| Lymph node negative | 39            | 37             | 76                 |
| Lymph node positive | 35            | 41             | 76                 |
| Primary tumours  | 37            | 41             | 78                 |
| Lymph node metastases | 40            | 33             | 73                 |
| ER positive      | 31            | 49             | 80                 |
| ER negative      | 37            | 37             | 74                 |
| HER2 negative    | 41d           | 35             | 76                 |
| HER2 positive    | 28d           | 46             | 74                 |
| Total            | 37            | 39             | 76                 |

CNA = copy number aberrations; WNY = Western New York. aP = 0.034. bP = 0.039. cP = 0.054. dP = 0.037.

Figure 2  HER2 amplification and overexpression. Nine tumours from eight patients showed amplification at 17q21. The chromosome 17 aCGH profiles of four of them are shown at the top along with one nonamplified sample (the y-axis is on a log2 scale). This was associated with Her2 protein overexpression by immunohistochemistry (middle) and Western blot analysis (bottom).
| BAC clone (RP11-) | Chr. loc. | #Samples (%) | #Samples with ≥3 copy gains | Genes |
|-----------------|-----------|--------------|-----------------------------|-------|
| 727M5           | 1p21.1    | 17 (36)      | 0                           |       |
| 3584            | 1q21.1    | 10 (21)      | 1                           | ABO33071, AF379633, AF379636, LOC64182, AK075065 |
| 439A17          | 1q21.1    | 9 (19)       | 1                           | BC034024 |
| 196G18          | 1q21.2    | 5 (11)       | 1                           |       |
| 307C12          | 1q22      | 5 (11)       | 1                           |       |
| 120D12          | 1q23.1    | 8 (17)       | 0                           | FCRH1, CDSL, AF329488 |
| 452O22          | 1q23.1    | 8 (17)       | 1                           | SPTA1, AF060556, M11049 |
| 520H16          | 1q23.1    | 7 (15)       | 0                           |       |
| 223F11          | 1q23.3    | 8 (17)       | 1                           | DDR2 |
| 403P14          | 1q23.3    | 8 (17)       | 0                           |       |
| 506O24          | 1q23.3    | 8 (17)       | 0                           |       |
| 572K18          | 1q23.3    | 8 (17)       | 0                           |       |
| 409E10          | 1q23.3    | 7 (15)       | 0                           | SLAMF1, CD48, SLAMF7 |
| 528G11          | 1q23.3    | 6 (13)       | 0                           |       |
| 80D6            | 1q24.1    | 6 (13)       | 0                           | LMX1A |
| 549H            | 1q24.1    | 5 (11)       | 0                           |       |
| 162H11          | 1q25.2    | 6 (13)       | 1                           | RASAL2 |
| 317P13          | 1q25.3    | 5 (11)       | 1                           | RGS16, RGS8 |
| 335O13          | 1q22.1    | 8 (17)       | 1                           | MPRRA4, MYOG, FMOD, MYBPH, CH1T |
| 148K15          | 1q22.1    | 6 (13)       | 1                           | MDM4, LRRN5 |
| 294K24          | 1q22.1    | 6 (13)       | 1                           | PTRN7, ARL10B |
| 150L7           | 1q22.1    | 5 (11)       | 0                           | PKP1, DKFZp434B1231 |
| 243M13          | 1q22.1    | 5 (11)       | 0                           | ABO18299, CNTN2 |
| 564A8           | 1q22.2    | 9 (19)       | 1                           | IL20, IL24, TOSO, MGCI4309, MGCP1GR, FKSG87, AY063126 |
| 167J2           | 1q22.2    | 8 (17)       | 0                           | AY320401 |
| 328D15          | 1q22.2    | 7 (15)       | 3                           | CD34 |
| 357P18          | 1q22.2    | 5 (11)       | 0                           |       |
| 35C1            | 1q22.2    | 5 (11)       | 1                           | CR1, CR2 |
| 211K12          | 1q41      | 5 (11)       | 0                           |       |
| 35D17           | 1q41      | 5 (11)       | 0                           | PTPN14 |
| 115K6           | 1q42      | 7 (15)       | 1                           |       |
| 112O22          | 1q43      | 6 (13)       | 0                           |       |
| 150L22          | 1q44      | 9 (19)       | 2                           | AKT3, SDCCAG8, BC019085 |
| 194A15          | 3q23.1    | 5 (11)       | 1                           | TIPARP |
| 88L18           | 5p15.1    | 5 (11)       | 0                           |       |
| 36H5            | 5p15.1    | 5 (11)       | 0                           | AK090679 |
| 438B9           | 6q26      | 8 (17)       | 1                           | LPA |
| 90P13           | 7p23.3    | 7 (15)       | 1                           |       |
| 331D5           | 7q36.3    | 11 (23)      | 4                           | DNAJB6, PTPRN2 |
| 83O14           | 8q12.23   | 9 (19)       | 2                           | ST18 |
| 182B11          | 8q12.3    | 8 (17)       | 1                           |       |
| 252M13          | 8q12.3    | 6 (13)       | 2                           |       |
| 566L6           | 8q13.2    | 23 (49)      | 0                           | ABO59542 |
| 120N14          | 8q13.2    | 5 (11)       | 1                           | SULF1 |
| 65P4            | 8q11.11   | 9 (19)       | 1                           |       |
| 203C23          | 8q11.11   | 6 (13)       | 0                           | LY96 |
| 53A24           | 8q11.11   | 5 (11)       | 1                           |       |
| 594N15          | 8q11.12   | 9 (19)       | 3                           | PKIA |
| 79J13           | 8q11.12   | 8 (17)       | 3                           | IL7, CGI-62 |
| 93J13           | 8q11.12   | 8 (17)       | 1                           |       |
| 523D2           | 8q11.12   | 7 (15)       | 3                           |       |
| 80C11           | 8q11.13   | 14 (30)      | 3                           | FABPS, BT007449 |
| 214E11          | 8q11.13   | 10 (21)      | 2                           | TP552 |
| 93E11           | 8q11.13   | 8 (17)       | 3                           |       |
| 219B4           | 8q12.1    | 5 (11)       | 1                           | E2FS, BC030701, AK056185 |
| 480D6           | 8q13.1    | 12 (26)      | 3                           | MMP16, DKFZp761D112 |
| 435I11          | 8q13.1    | 12 (26)      | 3                           | NBS1, DECR1, CBOr1 |
| 122C21          | 8q13.1    | 8 (17)       | 2                           | CBAA2T1 |
| 118O8           | 8q13.1    | 5 (11)       | 1                           | CBAA2T1 |
| 413N8           | 8q21.2    | 10 (21)      | 2                           | SDC2, AFI19386, AF107833 |
| 30H1            | 8q21.2    | 7 (15)       | 2                           | CDH17, RAD54B |
| 700M17          | 8q21.2    | 7 (15)       | 2                           | CDH17, GEM |
| 2715            | 8q21.2    | 6 (13)       | 2                           |       |
| 347C18          | 8q22.1    | 6 (13)       | 2                           | FL20530, TP53INPI |
| 498C11          | 8q22.1    | 5 (11)       | 2                           |       |
| 125O21          | 8q22.2    | 13 (28)      | 3                           | STK3, KCNS2, BC034778 |
| 131D12          | 8q22.2    | 7 (15)       | 1                           | COHI |
| 208E21          | 8q22.2    | 6 (13)       | 1                           |       |
| 580F3           | 8q22.3    | 6 (13)       | 1                           | ODF1, TIEG |
| 12K18           | 8q22.3    | 5 (11)       | 1                           |       |
cases. Two BAC clones with known genes were significantly more often amplified, and 13 were more frequently deleted in WNY tumours.

**DISCUSSION**

There is a paucity of data on genomic changes in early-onset breast cancer. The study reported here benefitted from a unique population, namely premenopausal women from Belarus who were exposed to significant fallout radiation from the 1986 Chernobyl nuclear accident. It has been suggested that Chernobyl-related cancers may be the best model to study the carcinogenic effect of low-dose radiation (Ermak et al., 1998; Albertson et al., 2002; Lage et al., 2003). We were able to compare the genomic abnormalities in the Belarusian cancers to those in similar tumours from a nonexposed area that were well matched for ethnic background, age, tumour characteristics, and extent of disease.

By utilising high-density aCGH technology with the capacity to screen for DNA copy changes across the entire genome at high resolution, we identified a larger number of chromosomal abnormalities than previous breast cancer studies. Links to genomic databases helped to identify candidate genes for further investigation. A small number of published studies applied aCGH technology to breast cancer cells, but only to a very limited extent (Pinkel et al., 1998; Albertson et al., 2000; Kauraniemi et al., 2001; Hyman et al., 2002; Pollack et al., 2002; Lage et al., 2003). Almost all of these utilised a significantly smaller number of specimens. Some of them focused only on cell lines (Hyman et al., 2002; Lage et al., 2003) or on individual chromosomes (Pinkel et al., 1998; Albertson et al., 2000; Kauraniemi et al., 2001). We are not aware of any published reports on array or conventional CGH analysis of DNA abnormalities in irradiated cells. Likewise, our study may be the first to screen for somatic genetic changes specifically in premenopausal tumours, providing new insight into the molecular pathology of early-onset breast cancer. The average number of total CNAs in our cohort of tumours was 76 (range 35–134), which is approximately eight times the average number of CNAs in breast cancers detected by conventional CGH (Isola et al., 1995; Kuukasjärvi et al., 1997; Aubele et al., 2000a, b; Zudaire et al., 2002), demonstrating the superior sensitivity of our assay. This is in keeping with the density of the BAC arrays used (some 3000 elements, average resolution ~1 Mb).

Several groups have used conventional CGH to probe for genetic aberrations in invasive breast carcinomas. Tirkkonen et al. (1998) found that the pattern of chromosomal gains and losses depended on tumour size, grade, and receptor status. They described gain on 1q as an early event and gain on 8q as a marker for advanced breast cancers. We have identified similar recurrent gains on 1q and 8q, which were the most commonly affected chromosomal arms in our series (Table 3). In a study of T2 (>2 cm) node-negative breast cancers, a high overall number of genetic aberrations was correlated with poor prognosis, and an increased copy number at 8q and 20q13 indicated an aggressive phenotype (Isola et al., 1995). Another similar study of invasive ductal carcinomas found that gains on 1q, 11q, 17q, and 20q were associated with poor prognosis (Zudaire et al., 2002). These DNA changes were common in our series as well, and this observation is in agreement with the more aggressive clinical course of premalignant breast cancer. Nine of 47 tumours showed amplification of a BAC clone including HER2. This copy gain rate (19%) is in keeping with numerous previous studies on HER2 amplification in breast cancer and proved to be among the most common abnormalities in our study. Additional commonly amplified BACs included the well-known breast cancer oncogenes c-myc (at 8q24.21) and cyclin D1 (at 11q13.3), further demonstrating adequate sensitivity of our method. Novel recurrent CNAs with potential target genes included gains at 3q25.31, 6q26, 7q36.3, 13q32.2, and 16p11.2 (Table 3). Seven recurrent amplification loci, most of which were found on 8q, had not been reported to be frequent events in breast carcinomas.

Recurrent deletions were less common, and no single event was found in more than 26% of the samples. Losses were most frequently observed on chromosome 17, which is in keeping with published cytogenetic data. However, nine of the remaining recurrent deletions, including seven on chromosome 22, had not been reported before (Table 4). Potential target genes included

---

**Table 3 (Continued)**

| BAC clone (RP11-) | Chr. loc. | #Samples (%) | #Samples with ≥3 copy gains | Genes |
|-------------------|-----------|--------------|----------------------------|-------|
| 17P14             | 17p13.11  | 3 (6)        | 0                          |       |
| 317F12            | 17p13.12  | 5 (11)       | 1                          |       |
| 468A19            | 17p13.12  | 5 (11)       | 1                          |       |
| 316E19            | 17q21.11  | 5 (11)       | 0                          |       |
| 316E19            | 17q21.11  | 5 (11)       | 0                          |       |
| 229L23            | 8q24.13   | 5 (11)       | 3                          | MYC   |
| 300I6             | 11q13.3   | 6 (13)       | 3                          | CCND1, FGF19 |
| 289E9             | 10p15.2   | 8 (17)       | 1                          |       |
| 120P20            | 11q13.3   | 13 (26)      | 4                          |       |
| 91P11             | 11q13.4   | 9 (19)       | 1                          |       |
| 461N23            | 13q22.2   | 6 (13)       | 1                          |       |
| 455F5             | 16p11.2   | 5 (11)       | 0                          |       |
| 521P1             | 17q12.1   | 5 (11)       | 1                          |       |
| 94L15             | 17q21.11  | 9 (19)       | 7                          |       |
| 109F13            | 20p11.23  | 5 (11)       | 0                          |       |
| 17D7              | 20q12     | 5 (11)       | 0                          |       |
| 145G103           | 20p13.12  | 5 (11)       | 0                          | PLTP, ZNF335, SLC12A5 |
| 560A15            | 20q13.31  | 5 (11)       | 1                          |       |
| 181G18            | 21q21.1   | 6 (13)       | 0                          |       |

Chr. loc. = chromosomal location. Bold abnormalities indicate previously undescribed recurrent gains. Shaded rows indicate the same chromosomal band.
KREMEN1 (on 22q12.1), which is a component of the Wnt signalling pathway (Mao et al., 2002).

It is conceivable that the novel chromosomal gains and losses described here may be the hallmark of premenopausal breast cancer. While our high-density BAC arrays appeared to have adequate sensitivity, an initial validation experiment suggested that the detected abnormalities may also be specific and verifiable by alternate techniques. The tumours with amplification of the

Table 4

| BAC clone (RP11-1) | Chr. loc. | #Samples (%) | #Samples with possible homozygous deletions | Genes |
|-------------------|-----------|---------------|---------------------------------------------|-------|
| 6B16              | 1p31.1    | 8 (17)        | 0                                           | TNRFSF8, TNRFSF18, BCO02167 |
| 23B013            | 1p34.3    | 5 (11)        | 0                                           | THRAP3 |
| 104J6             | 1p35.2    | 1             | 1                                           | FGR, GI3, MGC34648 |
| 406               | 1p35.3    | 5 (11)        | 1                                           | PTAER, DNAJC8, ATP1F1, SESN2 |
| 692D20            | 1p35.3    | 3 (6)         | 0                                           | MyL3, PTHR1, HYPR |
| 285H3             | 1p36.11   | 6 (13)        | 2                                           | MYL3, PTHR1, HYPR |
| 426M1             | 1p36.22   | 12 (26)       | 2                                           | TNRFSF8, TNRFSF18, BCO02167 |
| 21N6              | 2q24.3    | 10 (21)       | 0                                           | 1 |
| 425F              | 2q31.31   | 7 (15)        | 1                                           | MYL3, PTHR1, HYPR |
| 745J15            | 7p11.2    | 7 (15)        | 2                                           | PTPN3, CCT6A, SUMF2, PHKG1 |
| 148K1             | 7q36.1    | 9 (19)        | 0                                           | ACCN3, FASTK, ABCB8, ASB10, CDK5 |
| 89M8              | 8p21.2    | 7 (15)        | 1                                           | RHOB6T2, TNRFSF1OB |
| 23Q2              | 8p21.2    | 5 (11)        | 0                                           | BNP3L |
| 288N10            | 8p21.2    | 5 (11)        | 0                                           | 1 |
| 110I6             | 8p21.3    | 9 (19)        | 0                                           | 1 |
| 23S15             | 8p23.1    | 5 (11)        | 0                                           | GATA4, FDTI, NEIL2 |
| 240A7             | 8p23.3    | 11 (23)       | 0                                           | DLGAP2 |
| 18G9              | 8q21.2    | 8 (17)        | 1                                           | 1 |
| 22B815            | 9q34.11   | 9 (19)        | 0                                           | SH2D2C, CDK9, FPG2, ENG |
| 104F15            | 10q22.1   | 5 (11)        | 1                                           | LRRRC20, EFCGBP2, NODAL |
| 37L21             | 10q24.1   | 7 (15)        | 1                                           | 1 |
| 285H13            | 11q23.1   | 10 (21)       | 4                                           | TRPV3, CARKL, CTNS |
| 426M1             | 11q23.3   | 12 (26)       | 4                                           | TRPV3, CARKL, CTNS |
| 104H15            | 13q1.3    | 6 (13)        | 0                                           | 1 |
| 425F              | 13q1.3    | 7 (15)        | 0                                           | 1 |
| 23B124            | 14q32.33  | 5 (11)        | 2                                           | OIP5, ANKT, CFC30, ITPKA, LTK, KIAA0252 |
| 160F              | 15q1.1    | 6 (13)        | 2                                           | ZCH1C7, KIAA0513, BCO030280 |
| 12A11             | 15q1.1    | 5 (11)        | 0                                           | 1 |
| 1D5               | 17p13.1   | 5 (11)        | 0                                           | ALOX12B |
| 89A15             | 17p13.1   | 5 (11)        | 0                                           | RPL26, MYH10 |
| 231G16            | 17p13.2   | 11 (23)       | 2                                           | 1 |
| 20B12             | 17p13.2   | 12 (26)       | 4                                           | TRPV3, CARKL, CTNS |
| 61B16             | 17p13.3   | 11 (23)       | 1                                           | CT120, GEMIN4 |
| 216P6             | 17p13.3   | 9 (19)        | 0                                           | ABR, NXXN, TIMM22 |
| 26N16             | 17p13.3   | 7 (15)        | 0                                           | CGI-150, TIMM22 |
| 433M14            | 17p13.3   | 6 (13)        | 0                                           | 1 |
| 4F24              | 17p13.3   | 6 (13)        | 0                                           | RPA1 |
| 356L18            | 17p13.3   | 5 (11)        | 0                                           | 1 |
| 818O24            | 17p13.3   | 5 (11)        | 0                                           | 1 |
| 38B2              | 17q11.2   | 6 (13)        | 1                                           | CRL3F |
| 58C9              | 17q21.2   | 5 (11)        | 0                                           | GJCI, CTEN |
| 268D24            | 17q21.31  | 8 (17)        | 4                                           | P53E2, AOC2, AOC3, GSPC, RPL27, IFI35, AK055784 |
| 506G7             | 17q21.31  | 7 (15)        | 1                                           | 1 |
| 35Q16             | 17q21.32  | 10 (21)       | 0                                           | HOXB13, NOPS2, FJ35808 |
| 38BC12            | 17q25.3   | 7 (15)        | 0                                           | 1 |
| 12111             | 19p13.12  | 7 (15)        | 2                                           | TPM4, HSH2, AP1M1, KIP3, MEL, FLJ25328, DKFZP56801020, BT007184 |
| 56K21             | 19p13.12  | 5 (11)        | 2                                           | CD97, DDX39, PRKCL1 |
| 29BC17            | 19p13.2   | 8 (17)        | 0                                           | EIF34, DMT1, P2R1Y11 |
| 3IN2              | 19p13.2   | 6 (13)        | 1                                           | KIAA1198 |
| 38B2              | 19q13.2   | 9 (19)        | 1                                           | 1 |
| 21J5              | 19q13.3   | 7 (15)        | 1                                           | CALM3, GNG8, PTGIR |
| 236B14            | 19q13.33  | 7 (15)        | 0                                           | 1 |
| 17I20             | 19q13.33  | 5 (11)        | 2                                           | SULT2B1, RPL18, PHK2, DBP, CA11, FUT2 |
| 87L13             | 19q13.42  | 6 (13)        | 1                                           | 1 |
| 36N55             | 22q11.21  | 5 (11)        | 1                                           | SDF2L1 |
| 315L15            | 22q12.1   | 7 (15)        | 1                                           | KREMEN1 |
| 506               | 22q12.1   | 5 (11)        | 1                                           | 1 |
| 206B19            | 22q13.2   | 5 (11)        | 1                                           | SREBF2I |
| 49M21             | 22q13.31  | 7 (15)        | 1                                           | ARHGAPl8 |
| 61L22             | 22q13.31  | 6 (13)        | 0                                           | SMCL2, FLJ20635 |
| 11132             | 22q13.33  | 5 (11)        | 0                                           | MLC1, MOV10L1, PANX2 |

Chr. loc. = chromosomal location. Bold abnormalities indicate previously undescribed recurrent losses. Shaded rows indicate the same chromosomal band.
HER2 locus also showed overexpression of the Her2 oncoprotein, while the nonamplified tumours showed no immunoreactivity (Figure 2).

Our series included five matched pairs of primary tumours and nodal metastases from Belarus. As expected, a number of chromosomal abnormalities were common to both the metastasis and the parental tumour, although the number of shared CNAs varied significantly from case to case (Table 5). Importantly, all cases were characterised by a large number of discordant events. Primary tumours developed a smaller, larger, or similar number of CNAs compared to the secondary lesions. They tended to have more deletions than losses. In contrast, nodal metastases consistently were marked by a larger number of amplifications over deletions. These findings suggest that metastases may form at variable points in the evolution of a breast cancer, and that primary tumour and metastasis independently develop additional genetic changes. This adds to the growing body of evidence that metastatic breast cancers may be biologically distinct from their parental tumours.

As detailed in Table 2, the genetic changes were not evenly distributed among the premenopausal breast cancers. While chromosomal gains and losses were not dependent on tumour site or size, nodal involvement, or ER status, younger age and HER2 negativity were associated with a smaller number of deletions and more amplifications. Likewise, tumours from Belarus had more DNA gains and fewer deletions than carcinomas from WNY. One of the most interesting observations was that breast cancers from Gomel had a DNA profile that was distinct from that of an age- and stage-matched control group treated at RPCI. Strikingly, when all 47 cases were subjected to unsupervised hierarchical clustering, two distinct groups emerged: one mostly comprised of Belarussian breast cancers and one mainly from WNY (Figure 3). This segregation was statistically highly significant ($P < 0.001$). All of the other variables (age, extent of disease, grade, receptor status) were similarly distributed in the two arms. In all, 50 BAC clones were differentially amplified or deleted in the two groups of tumours, and half of these contained named genes. In total, 10 chromosomal gains were specific to the Belarussian samples, and it is tempting to speculate that these may represent the molecular hallmark of radiation associated breast cancer. Potential target genes included the MDM2-related gene MDM4 (at 1q32.1) and SULT1A3 (at 16p11.2) encoding an enzyme involved in the metabolism of catecholamines and related compounds (Hildebrandt et al., 2004).

WNY tumours were characterised by a significantly higher number of deleted BAC clones. It is unclear whether the chromosomal

| Paired case # | Gains | Losses | Gains | Losses | Gains | Losses |
|--------------|-------|--------|-------|--------|-------|--------|
| 1            | 63 (33) | 9 (7) | 16 (11) | 9 (8) | 30 (25) | 12 (11) |
| 2            | 19 (13) | 10 (7) | 21 (15) | 26 (25) | 31 (30) | 15 (13) |
| 3            | 20 (11) | 1 (1) | 48 (38) | 37 (30) | 42 (35) | 22 (20) |
| 4            | 7 (5) | 9 (5) | 25 (20) | 34 (31) | 36 (33) | 24 (23) |
| 5            | 13 (7) | 0 (0) | 13 (12) | 91 (63) | 42 (29) | 4 (3) |

Number of amplified or deleted BAC clones (number of affected chromosomal bands in parentheses).
abnormalities in the tumours from Gomel are important in their pathogenesis or merely markers of genomic susceptibility to radiation damage. There are no associated epidemiologic or dosimetry data for the individual samples from Belarus so that, at this juncture, we cannot be sure that the specific changes in the tumour DNA are in fact due to radiation exposure. While the Belarussian population is ethnically homogeneous (Ermak et al., 2003), and while the racial background of the breast cancer patients from the Gomel area was similar to that in the WNY cohort, we cannot rule out that other endogenous or exposure variables such as smoking or diet may account for the difference in genomic abnormalities, although there is no evidence that such variables such as smoking or diet may account for the difference in genomic abnormalities, although there is no evidence that such environmental factors impact on the pattern of chromosomal aberrations in breast carcinomas.

In conclusion, our study significantly adds to the existing body of knowledge by (a) detailing a number of previously undescribed recurrent chromosomal gains and losses in premenopausal breast cancers; (b) focusing on a unique cohort of breast carcinomas associated with prolonged low-dose radiation exposure; and (c) describing a set of CNAs that are specific to tumours from a nuclear fallout area. Our findings may provide the basis for future studies on the molecular pathogenesis of early-onset breast cancer.

ACKNOWLEDGEMENTS

This work was supported in part by Grant No. N00014-94-1-0049, issued to Georgetown University from the Office of Naval Research in support of the International Consortium for Research on the Health Effects of Radiation. The contents are solely the responsibility of the authors and do not represent the views of the Office of Naval Research, Georgetown University, or the International Consortium for Research on the Health Effects of Radiation. This study was also supported by shared resources of the Roswell Park Cancer Center Support Grant P30 CA16056 and by a UICC International Cancer Technology Transfer Fellowship (to A. Pryschepava).

REFERENCES

Albertson DG, Ylstra B, Seagraves R, Collins C, Dairkee SH, Kowbel D, Kuo WL, Gray JW, Pinkel D (2000) Quantitative mapping of ampiclon structure by array CGH identifies CYP24 as a candidate oncogene. Nat Genet 25: 144 – 146
Aubele M, Cummings M, Walsch A, Zitzelsberger H, Nahrig J, Holfer H, Werner M (2006a) Heterogeneous chromosomal aberrations in intraductal breast lesions adjacent to invasive carcinoma. Anal Cell Pathol 29: 17 – 24
Aubele MM, Cummings MC, Mattis AE, Zitzelsberger HF, Walch AK, Kremer M, Holfer H, Werner M (2006b) Accumulation of chromosomal imbalances from intraductal proliferative lesions to adjacent in situ and invasive ductal breast cancer. Diagn Mol Pathol 15: 14 – 19
Behrens G, Travis LB, Wistuba II, Davis S, Maitra A, Clarke EA, Lynch CF, Glimelius B, Wiklund T, Tarone R, Gazdar AF (2000) Molecular changes in second primary lung and breast cancers after therapy for Hodgkin’s disease. Cancer Epidemiol Biomarkers Prev 9: 1027 – 1035
Clemens M, Loijens L, Goss P (2000) Breast cancer risk following irradiation for Hodgkin’s disease. Cancer Treat Rev 26: 291 – 292
Cowell JK, Matsui S, Wang YD, LaDuca J, Conroy J, McQuaid D, Nowak NJ (2004a) Application of bacterial artificial chromosome array-based comparative genomic hybridization and spectral karyotyping to the analysis of glioblastoma multiforme. Cancer Genet Cytogenet 151: 36 – 51

Table 6 Genetic changes distinguishing premenopausal breast cancers from WNY and Gomel, Belarus

| BAC clone (RP11-) | Chr. loc. | Candidate genes | Ampl. | Del. | Ampl. | Del. |
|------------------|----------|-----------------|--------|------|--------|------|
| 425L11 | 8q21.3 | DECR1, C8orf1 | 9 | 2 | 6 | 2 |
| 122C21 | 8q21.3 | CBFAP2T1 | 0 | 0 | 0 | 0 |
| 157P1 | 2q13.33 | OSMPL2, ADRM1, LAMAS | 0 | 0 | 0 | 0 |
| 148K15 | 1q32.1 | MDM4 | 0 | 0 | 0 | 0 |
| 461N23 | 1q32.3 | EB2, GPR18 | 0 | 0 | 0 | 0 |
| 150L7 | 1q32.1 | PKPI | 0 | 0 | 0 | 0 |
| 243M13 | 1q32.1 | CNTN2, RBBP5, ABO18299 | 0 | 0 | 0 | 0 |
| 455F5 | 1p11.2 | SULT1A3, ALDOA, TBX6, CORO1A, MGC5178 | 0 | 0 | 0 | 0 |
| 465L10 | 20q13.12 | PLTP, ZNF335, SLCol1A5, NCOA5 | 0 | 0 | 0 | 0 |
| 252A24 | 16q22.33 | PSMD7, GLGI | 0 | 4 | 3 | 1 |
| 216 | 5q35.3 | COL2A1 | 1 | 4 | 3 | 0 |
| 58M17 | 16p13.2 | USP7 | 0 | 3 | 4 | 0 |
| 36F15 | 17q11.2 | CRLF3 | 6 | 0 | 0 | 0 |
| 126L15 | 7q22.1 | ZAN, EPHH4, ACHE | 3 | 0 | 0 | 0 |
| 211E17 | 11p15.4 | TRIM3, ILK, TAF10, CLN2, PCDH16 | 3 | 0 | 0 | 0 |
| 35J17 | 19q13.42 | ZNF331 | 3 | 0 | 0 | 0 |
| 571M6 | 12q14.1 | CDK4 | 3 | 0 | 0 | 0 |
| 746M1 | 17p11.2 | USP22, DKFZp5660084, C17orf35 | 3 | 0 | 0 | 0 |
| 36J16 | 17q21.32 | NDRG2, HOXB13 | 8 | 2 | 2 | 2 |
| 201J12 | 17p13.2 | TRVI, CARKL, CTNS | 8 | 2 | 2 | 2 |
| 61B26 | 17p13.3 | CT120, GEMIN4 | 8 | 3 | 3 | 3 |
| 213L15 | 22q12.1 | KREMEN1 | 6 | 1 | 1 | 1 |
| 2J15 | 19q13.32 | CALM3, PTGIR, GNG8 | 6 | 1 | 1 | 1 |
| 89M8 | 8p21.2 | RHOB1N2, TNFRSF10B | 6 | 1 | 1 | 1 |
| 29B17 | 19p13.2 | EIF354, DNM1T1, FPRY11 | 6 | 1 | 1 | 1 |

WNY = Western New York; Chr. loc. = chromosomal location; Ampl. = amplification; Del. = deletion. In all, 25 (out of 50) distinguishing BAC clones contained named genes (listed). Ten BAC clones (bold) were selectively amplified in Belarussian breast cancers and may represent signature events related to radiation exposure.
Cowell JK, Nowak NJ (2003) High-resolution analysis of genetic events in cancer cells using bacterial artificial chromosome arrays and comparative genome hybridization. *Adv Cancer Res* **90:** 91–125

Cowell JK, Wang YD, Head K, Conroy J, McQuaid D, Nowak NJ (2004b) Identification and characterisation of constitutional chromosome abnormalities using arrays of bacterial artificial chromosomes. *Br J Cancer* **90:** 860–865

Durante M, Grossi GF, Yang TC (1996) Radiation-induced chromosomal instability in human mammary epithelial cells. *Adv Space Res* **18:** 99–108

Ermak G, Figge JJ, Kartel NA, Davies KJA (2003) Genetic aberrations in Chernobyl-related thyroid cancers: Implications for possible future nuclear accidents or nuclear attacks. *IUBMB Life* **55:** 637–643

Gaffney DK, Hammersmeier J, Holden J, Marshall J, Smith LM, Avizonis V, Tran T, Neuhausen SL (2001) Breast cancer after mantle irradiation for Hodgkin’s disease: correlation of clinical, pathologic, and molecular features including loss of heterozygosity at BRCAl and BRCA2. *Int J Radiat Oncol Biol Phys* **49:** 539–546

Hildebrandt MA, Salavaggione OE, Martin YN, Flynn HC, Jalal S, Wieben ED, Weinshilboum RM (2004) Human SULT1A3 pharmacogenetics: gene duplication and functional genomic studies. *Biochem Biophys Res Commun* **321:** 870–878

Hyman E, Kauraniemi P, Hautaniemi S, Moul M, Rouzenblum E, Ringner M, Sauter G, Monni O, Elkahlon A, Kallioniemi OP, Kallioniemi A (2002) Impact of DNA amplification on gene expression patterns in breast cancer. *Cancer Res* **62:** 6240–6245

Isola J, Kallioniemi OP, Chu LW, Fuqua SA, Hilsenbeck SG, Osborne CK, Waldman FM (1995) Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. *Am J Pathol* **147:** 905–911

Kauraniemi P, Bärlund M, Monni O, Kallioniemi A (2001) New amplified and highly expressed genes discovered in the ERBB2 amplicon in breast cancer by cDNA microarrays. *Cancer Res* **61:** 8235–8240

Kuukasjärvi T, Karhu R, Tanner M, Kahkonen M, Schaffer A, Nuppenon N, Pennanen S, Kallioniemi A, Kallioniemi OP, Isola J (1997) Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer. *Cancer Res* **57:** 1597–1604

Lage JM, Leamon JH, Pejovic T, Hamann S, Lacey M, Dillon D, Segraves R, Vossbrinck B, Gonzalez A, Pinkel D, Albertson DG, Costa J, Lizardi PM (2003) Whole genome analysis of genetic alterations in small DNA samples using hyperbranched strand displacement amplification and array-CGH. *Genome Res* **13:** 294–307

Land CE, Tokunaga M, Koyama K, Soda M, Preston DL, Nishimori I, Tokuoka S (2003) Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950–1990. *Radiat Res* **160:** 707–717

Mahoney MC, Lawvere S, Falkner KL, AverIn YL, Ostapenko VA, Michalek AM, Moysich KB, McCarthy PL (2004) Thyroid cancer incidence trends in Belarus: examining the impact of Chernobyl. *Int J Epidemiol* **33:** 1025–1033

Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, Delius H, Hoppe D, Stannek P, Walter C, Glinka A, Niehrs C (2002) Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signaling. *Nature* **417:** 664–667

Peterson LE, Dreyer ZE, Plon SE, Smith JL, Weinberg A, McCarthy P (1997) Design and analysis of epidemiological studies of excess cancer among children exposed to Chernobyl radionuclides. *Stem Cells* (Suppl. 2): 211–230

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* **20:** 207–211

Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Borresen-Dale AL, Brown PO (2002) Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc Natl Acad Sci USA* **99:** 12963–12968

Roy D, Calaf G, Hei TK (2001) Profiling of differentially expressed genes induced by high linear energy transfer radiation in breast epithelial cells. *Mol Carcinogen* **31:** 192–203

Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Storn A, Snuffin M, Rezants E, Popov D, Byfluow A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V, Quackenbush J (2003) TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* **34:** 374–378

Sasnouskaya A, Okeanov A (2000) Breast cancer incidence in Mogilev Oblast after the Chernobyl accident. *Zdrowoohranenije (Publ Health)* **10:** 26–28

Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle AK, Huey B, Kinmura K, Law S, Myambo K, Palmer J, Ylstra B, Yue JP, Gray JW, Jain AN, Pinkel D, Albertson DG (2001) Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* **26:** 263–264

Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J, Kallioniemi OP (1998) Molecular cytogenetics of primary breast cancer by CGH. *Genes Chromosom Cancer* **21:** 177–184

Travis LB, Hill DA, Dores GM, Gospodarowicz M, van Leeuwen FE, Holowaty E, Glimelius B, Andersson M, Wiklund T, Lynch CF, Van’t Veer MB, Glimelius I, Storm H, Pukkala E, Stovall M, Curtis R, Boice Jr JD, Gilbert E (2003) Breast cancer following radiotherapy and chemotherapy among young women with Hodgkin disease. *JAMA* **290:** 465–475

Tuttle RM, Becker DV (2000) The Chernobyl accident and its consequences: update at the millennium. *Semin Nucl Med* **30:** 133–140

Wazer DE, Chu Q, Liu X-L, Gao Q, Safali H, Band V (1994) Loss of p53 protein during radiation transformation of primary human mammary epithelial cells. *Mol Cell Biol* **14:** 2466–2478

Yang TC, Georgy KA, Craise LM, Durante M, Grossi GF, Yang TC (1996) Radiation-induced chromosomal instability in human mammary epithelial cells. *Adv Space Res* **18:** 99–108

Zudaire I, Odero MD, Caballero C, Valenti C, Martinez-Penuela JM, Isola J, Calasanz MJ (2002) Genomic imbalances detected by comparative genomic hybridization to microarrays. *Radiat Oncol Invest* **5:** 134–138

Zudarle I, Odero MD, Caballero C, Valenti C, Martinez-Penuela JM, Isola J, Calasanz MJ (2002) Genomic imbalances detected by comparative genomic hybridization to microarrays. *Radiat Oncol Invest* **5:** 134–138

Zudarle I, Odero MD, Caballero C, Valenti C, Martinez-Penuela JM, Isola J, Calasanz MJ (2002) Genomic imbalances detected by comparative genomic hybridization to microarrays. *Radiat Oncol Invest* **5:** 134–138