Gene copy number variation in male breast cancer by aCGH

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Abstract. *Background:* Male breast cancer (MBC) is a rare disease and little is known about its etiopathogenesis. Array comparative genomic hybridization (aCGH) provides a method to quantitatively measure the changes of DNA copy number and to map them directly onto the complete linear genome sequences. The aim of this study was to investigate DNA imbalances by aCGH and compare them with a female breast cancer dataset.

Methods: We used Agilent Human Genome CGH Microarray Kit 44B and 44K to compare genomic alterations in 25 male breast cancer tissues studied at NCC of Bari and 16 female breast cancer deposited with the Gene Expression Omnibus (GSE12659). Data analysis was performed with Nexus Copy Number 5.0 software.

Results: All the 25 male and 16 female breast cancer samples displayed some chromosomal instability (110.93 alterations per patient in female, 69 in male). However, male samples presented a lower frequency of genetic alterations both in terms of loss and gains.

Conclusion: aCGH is an effective tool for analysis of cytogenetic aberrations in MBC, which involves different biological processes than female. Male most significant altered regions contained genes involved in cell communication, cell division and immunological response, while female cell–cell junction maintenance, regulation of transcription and neuron development.

Keywords: aCGH, male breast cancer, familiarity

1. Introduction

Male breast cancer (MBC) is a rare pathology representing about 1% of breast cancers (BC) [9]. The prevalence of MBC increases with age and its susceptibility may result from rare mutations in high penetrance genes such as BRCA1 and BRCA2, in particular BRCA2 (4–40%) more than BRCA1 (4%) mutations [8]. Other genes seem to be involved in MBC such as PTEN which results mutated in 22% [31], and other low penetrance genes such as CHEK2, mutated in 9% of MBC cases [8]. In families with CHEK2*1100delC variant, the risk of MBC was reported as ten times greater than normal [8]. Interestingly, the contribution

of the CHEK2*1100delC mutation to BC predisposition varies by ethnic group and from country to country [4,14].

In spite of the above mentioned genes seem to confer susceptibility to functional MBC, the genetic causes of this pathology are not well known. To date, the reports documenting genome-wide copy number changes by CGH on metaphase in MBC have shown similarity in the pattern of imbalances with respect to the chromosomal alterations described in female breast cancers (FBC) [1,17,25] suggesting a common etiology for the disease process in both sexes. Moreover, many of the genetic aberrations shared by MBC and FBC are common to other epithelial cancer types [10]. This is surprising, considering the different hormonal milieu in which the tumor develops and the established role of estrogens in promoting BC development and progression [5].

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Array Comparative Genomic Hybridization (aCGH) is a technique for obtaining an alteration profile in terms of gain and loss of chromosomal regions [20,24].

Since the resolution of the arrays has improved over the years, array CGH has become a powerful tool. It permits high-resolution detection of DNA copy number imbalances, as well as to map such copy number alterations throughout the genome besides the identification of genes mapped at the amplified/lost chromosomal regions and the frequency of alterations of specific genes involved in hereditary breast cancer [16].

The computational analysis of the experimental data involves the preprocessing of raw microarray data, aligning data with genome location, identification of altered chromosomal segments, assignment of a copy number state to each segment and highlighting of regions repetitively changed across several experiments.

The present paper focuses on the characterization of DNA imbalances in a set of 25 MBC samples analyzed by high-resolution CGH arrays in order to detect DNA copy number aberrations (CNAs). These results have been then compared with a female breast cancer dataset deposited with the Gene Expression Omnibus.

2. Materials and methods

2.1. Patients enrolment

Male patients affected by breast cancer have been consecutively selected for this retrospective study: 20 received the primary diagnosis of breast cancer at the NCC of Bari, while 5 at Regina Elena Institute of Rome, Italy.

Patients were informed about the aims and limits of this type of study before giving their written consent. DNA was extracted from peripheral blood and mutation analysis for whole BRCA1 and BRCA2 genes was performed utilizing dHPLC as a pre-screening analysis and automatic DNA sequencing for the identification of specific alterations, as previously reported [27]. All but 3 patients failed to show any abnormality in the BRCA1 and BRCA2 genes, one had germline mutation in BRCA1 and two in BRCA2.

The mean age of the patients at diagnosis was 58 years (range 38–78). All the tumors were invasive carcinomas and none of the carcinomas had been treated by chemotherapy or radiation therapy prior to resection. aCGH analysis was performed on the DNA from fresh frozen tumor tissues.

2.2. DNA extraction and aCGH analysis

Genomic DNA was extracted from 20 mg of frozen tumor tissues using the DNeasy tissue kit (Qiagen). Before extracting DNA, specimens underwent macrodissection on the basis of a specular specimen coloured by HE and controlled by a pathologist to present more than 70% of neoplastic cells. The reactions were checked on 0.8% agarose gel and the DNA obtained was quantified by spectrophotometry (Nanodrop, Celbio).

aCGH was performed using the Agilent Human Genome CGH Microarray Kit 44B and the Agilent Human Genome CGH Microarray Kit 44K (Agilent Technologies, Santa Clara, CA, USA). Data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE23891 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23891>).

Both the used platforms are high-resolution oligonucleotide based microarrays that allow genome-wide survey and molecular profiling of genomic aberrations with a resolution of about 100 kb. Labelling and hybridization were performed following the protocols provided by Agilent. Briefly, 500 ng of purified DNA of a patient and of a control were double-digested with *RsaI* and *AluI* for 2 h at 37°C. After 20 min at 65°C, DNA of each digested sample was labelled by the Agilent random primers labelling kit for 2 h using Cy5-dUTP for the patient DNA and Cy3-dUTP for the control DNA. Labelled products were column purified and prepared according to the Agilent protocol. After probe denaturation and pre-annealing with 50 µg of Cot-1 DNA (Invitrogen, Carlsbad, CA, USA), hybridization was performed at 65°C with rotation for 40 h. After two washing steps, the arrays were analyzed with the Agilent scanner and the Feature Extraction software (v8.0; v9.1.3). Graphical overview was obtained using the CGH analytics software (v3.1; v3.4).

2.3. Microarray data analysis

Array-based CGH data analysis was performed with Nexus Copy Number 5.0 software (Biodiscovery, Inc., El Segundo, CA, USA). This software uses the Rank Segmentation algorithm, a proprietary variation much faster at processing, on Circular Binary Segmentation (CBS) developed by Adam Olsen [18], together with the statistical Significance Testing for Aberrant Copy number (STAC) method, developed at the Penn Center for Bioinformatics at the University of Pennsyl-

vania, to identify non-random genomic amplifications and deletions across multiple experiments [6].

We used the modified CBS algorithm to improve the processing speed. It uses a normal distribution function to test for changing points as opposed to the original algorithm based on non-parametric permutation. It is a recursive algorithm that keeps dividing the genome into smaller and smaller segments until no region can be further segmented. The result is to segment the genome into clusters of uniform ratios. The algorithm has a single parameter called Significance Threshold that controls if a region is to be segmented out or not. At the completion of the segmentation process, the entire genome can be represented as a series of segments and each segment having a cluster value which is the median log-ratio value of all the probes in that region. The calling algorithm then uses the cluster values and the user defined threshold to establish regions of copy number variations.

According to this algorithm, two regions are considered different when p -values are lower than $1.0E-6$. Genomic regions of gains are defined as averaged $\log_2$ CGH fluorescence ratio ≥ 0.2 and losses as averaged $\log_2$ CGH ratio ≤ -0.2 . $\log_2$ ratios ≥ 0.6 are considered as high copy number gains and $\log_2$ ratios ≤ -1 as big copy number losses.

Frequency significance testing, instead, helps to identify the areas of the genome where there is a statistically significant high frequency of aberrations over the “background” level. The algorithm identified a set of aberrations that are stacked on top of each other such that it would not occur randomly. To find these events, the aberrations were permuted in each arm of each chromosome to see how likely it was for an event to occur at any location at a particular frequency. We have used a p -value cut-off of 0.05 and highlighted those areas that met the given p -value.

To attain a further biological knowledge about the aberrant regions of our samples we performed an enrichment analysis on the selected regions. The enrichment analysis identified GO terms that were significantly overrepresented and the genes annotated with these terms within the aberrant region. To perform this, we have considered two p -value measures: the standard p -value, where the probability of a particular gene being present in the set was treated independently of all others, and the Markov process p -value (MP p -value), a BioDiscovery proprietary algorithm that takes into account the relative location of the genes in the genome when estimating the probability. Each chromosome was modeled as a Markov process which

allows the p -value to take into account how close the genes were on the chromosome such that a cluster of genes participating in a single event will not get as high a significance value.

To integrate our analysis, we compared our dataset with a female breast cancer dataset (GSE12659) deposited with the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12659>), to which the same algorithms have been applied [32]. Among datasets available online this has been chosen being, in our opinion, the most comparable to ours in terms of number of cases and platform of analysis. Our aim was to evidence any chromosomal regions differently altered in female and male breast cancer even if female dataset contains all basal like breast cancer while male dataset has 80% ER and 60% HER-2 positive.

3. Results

All 25 male and 16 female breast cancer samples (GSE12659) displayed some chromosomal instability (Fig. 1). However, female presented a higher frequency of genetic alterations both in terms of loss and gains. These data are summarized in Table 1. Each male patient presented an average rate of 68 aberrations. Amplifications were prominent in chromosome 7 (50%), 11 (50%), 16 (40%) and X (70%). Chromosomal deletions were more frequent on chromosome 1 (60%), 2 (70%), 4 (50%), 5 (40%), 14 (53.33%), 15 (46.66%), 19 (40%), Y (40%). The aberrations were unequally distributed among patients, with four of them presenting less than ten aberrations. The number of aberrations did not seem to depend on the age of the patients, since patients 15 and 24, the youngest of the group (38 years old), have 83 and 66 aberrations respectively, while patients 1, 3, 4 and 9 with the fewest number of aberrations are 54, 71 and two are 63 years old.

In the female dataset (GSE12659), an average of 110.93 aberrations has been found: amplifications were more recurrent on chromosome 1 (85.5%), 2 (68.75%), 3 (68.75%), 8 (43.75%), 11 (56.25%), 17 (56.25%), 20 (50%). Deletions were more frequent in chromosome 2 (43.75%), 3 (62.5%), 7 (50%), 15 (81.25%), 16 (50%), 17 (68.75%).

Table 2 shows the specific chromosomal regions more frequently altered between male and female patients ($p < 0.05$). Regions 2p22.3 and 15q15.1 seemed to be significantly highly frequent in both male and female datasets; while, all other evidenced re-

Fig. 1. Frequencies of copy number gains and losses across the male and female genome. Copy number gains (green) and losses (red) are mapped relatively to their position. (The colors are visible in the online version of the article; <http://dx.doi.org/10.3233/ACP-CLO-2010-0544>.)

gions resulted specifically altered in male or in female group.

3.1. Hereditary breast cancer genes

Since male breast cancer may be considered to present hereditary characteristics, the frequency of alterations of specific genes involved in hereditary breast cancer susceptibility has been investigated. Frequency of alteration of these genes in the 2 datasets is reported in Table 3. BRCA2 resulted more deleted in male than in female series (50 vs. 25%, respectively), while BRCA1 was more frequently amplified in male group (30 vs. 12%, respectively). ATM, CHEK1, CHEK2 and PgR genes never resulted amplified in male BC, while ERBB2, KLF6, KLF10 and MYC never resulted deleted. ERBB2 was amplified in 30% of samples and MYC in 53.33%.

3.2. Gene ontology analyses

The enrichment analysis to identify GO terms significantly overrepresented in the aberrant regions evidenced different biological processes preferentially activated in male and female ($p < 0.01$): the most significant altered regions in male contained genes involved in cell communication, cell division and immunological response, while cell-cell junction maintenance, regulation of transcription and neuron development in female.

4. Discussion

We used high-resolution CGH microarrays to profile chromosomal aberrations in a set of MBC samples. It is well known that, as occurs in most solid tumors, MBC progression to malignancy is the result of a multistep process in which genomic DNA alteration plays a very important role, affecting gene expression of downstream key cellular processes. The application of DNA microarray technology to the conventional CGH technologies permits to achieve a reliable high-resolution mapping of unbalanced genetic alterations, including DNA gains and losses [30]. Moreover, the identification of the impact of DNA copy number aberrations on gene expression allows new knowledge on the pathology of these tumors as well as it opens the way to the detection of new diagnostic and/or prognostic markers [2,13].

We conducted the analysis on the whole genome to better evidence the genomic regions involved in breast carcinogenesis and, in particular, in hereditary breast cancer. We compared the findings on our series (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23891>) with those found on a female breast cancer dataset deposited with the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12659>) [32].

An average of 69 aberrations were found in the male tumors strengthening the role of chromosomal instability even if less than in the female series (mean = 111).

Table 1

Chromosomal regions more frequently altered in male and female breast cancer

Cytoband location	Event	Frequency (%)
Male breast cancer (NCC Bari, GSE23891)		
1p35.3	CN loss	60
2p22.3	CN loss	70
4p14	CN loss	50
5q13.1–q13.2	CN loss	40
7q36.1	CN gain	50
11p15.5	CN gain	50
11q13.1–q13.2	CN gain	50
14q13.1–q13.2	CN loss	53.33
15q15.1	CN loss	46.66
15q22.31	CN loss	46.66
16p11.2–p11.1	CN gain	40
19q13.11	CN loss	40
Xp11.23	CN gain	70
Yq11.222–q11.223	CN loss	40
Yq11.1–q11.221	CN loss	40
Female breast cancer (GSE12659)		
1q23.2–q23.3	CN gain	87.5
2p22.3	CN loss	43.75
2q31.1	CN gain	68.75
2q33.1–q33.2	CN loss	43.75
3p21.31	CN loss	62.5
3q29	CN gain	68.75
7p22.1	CN loss	50
8p11.21	CN gain	43.75
11q24.3	CN gain	56.25
15q15.1–q21.1	CN loss	81.25
16q22.1	CN loss	50
17q11.2	CN loss	68.75
17q24.3	CN gain	56.25
20q13.32–q13.33	CN gain	50
20q13.33	CN gain	50

Table 2

Different number of alterations in male and female datasets

	Loss	Gain	Homozygous loss	High copy gain	Mean of alterations per patient
Male	356	380	3	112	69
Female	738	804	5	228	110.93

An equal distribution between gain and loss regions was present pointing towards a high role for both oncogenes activation and tumor suppressor genes deactivation in MB carcinogenesis. MBC alterations targeted chromosomes 1, 2, 4, 7, 11, 14 and X in more than 50% of the cases, while chromosomes 1, 2, 3, 7, 11, 15, 16,

17 and 20 were altered in more than 50% of the female series. Our data strengthens the reports of Rudlowski et al. [21], who found similar imbalances between male and female BC with a less sensitive CGH.

CNAs have been demonstrated to determine cancer predisposition in most high risk cancer syndromes. Among CNAs alterations we focused our attention on those belonging to regions including genes usually linked to breast cancer biology [14].

MBC predisposition may result from germline mutations in BRCA2 and, at lower extent, in BRCA1 genes [26]. The frequency of BRCA1/BRCA2 mutations ranges from 4 to 40% for BRCA2 and up to 4% for BRCA1, being higher in the presence of founder effects [9,19,27]. Although BRCA1/BRCA2 mutations are more frequent among MBC cases with a positive BC family history (FH), they have also been reported among FH-negative MBC patients [10,11].

A somatic loss of the remaining BRCA wild-type allele confers the malignant breast cancer phenotype. As expected in our series, somatic BRCA2 was deleted in 50% of samples, much more than BRCA1 (20%). The large percent of copy number aberrations of BRCA2 observed in tumor cells supports the hypothesis that the protein product is involved in maintaining appropriate chromosome segregation and/or chromosomal repair. The high number of genetic defects detected in BRCA2 carriers indicates that germline mutation of these genes results in an accelerated accumulation of secondary somatic genetic changes in the tumors. This acceleration could explain the aggressive phenotype of tumor growth in tumors from BRCA2 carriers, as breast cancers associated with the BRCA2 mutation are high grade tumors with a rapid proliferation rate [1]. The chromosome defects in BRCA2 tumors are likely to be helpful in the understanding of the somatic genetic progression pathways that contribute to the development of malignancy in genetically predisposed individuals.

Several studies identify PALB2 as a moderate penetrance breast cancer susceptibility gene [22,23], accounting for about 1% of BRCA1/BRCA2 negative familial early onset BCs. Loss of somatic PALB2 locus resulted two folds higher in our MBC series than in FBC series suggesting that this gene may be specifically involved in MBC risk.

Truncating and missense mutations in the ATM gene, which cause insufficient DNA damage surveillance, allow damaged cells to proceed into mitosis, which possibly results in increased cancer susceptibility. Various missense mutations in ATM have been associated with breast cancer in case-control studies [7].

Table 3
Hereditary breast cancer genes alterations

Gene	Chromosome band	Male		Female	
		Amplified (%)	Deleted (%)	Amplified (%)	Deleted (%)
BRCA1	17q21	30	20	12	31.25
BRCA2	13q12.3	10	50	6.25	25
ATM	11q22–q23		40	12	31.25
CHEK1	11q24–q24		30	0.8	12.05
CHEK2	22q12.1		50	25	37.05
ERBB2	17q21.1	30		12.50	31.25
ESR1	6q25.1	13.33	60	0.8	25
KLF6	10p15	20		55.80	6.25
KLF10	8q22.2	40		75	
MYC	8q24.21	53.33		87.25	
PALB2	16p12.2	30	13	31.25	6.25
PgR	11q22–q23		40	25	
TP53	17p13.1	10	26.67		43.75

In our series ATM is deleted in 40% of samples, according with the increased cancer susceptibility.

Amplification of the two oncogenes ERBB2 and C-MYC and inactivation of the tumor suppressor gene P53 are frequent in breast carcinomas [1]. In the present series of MBC, the amplification of ERBB2 in 30% of patients, of MYC in 53.33% and deletion in 26.67% of TP53 was then expected, confirming the role of the two oncogenes and of the tumor suppressor in human breast carcinogenesis.

Mutation of the CHEK2 gene has also been associated with a high risk of breast cancer, especially MBC; CHEK2 encodes a cell-cycle checkpoint kinase implicated in DNA repair processes involving BRCA1 and p53 [15,28]. A study by the CHEK2 Breast Cancer Consortium observed that CHEK2*1100delC, a truncating variant that abolishes the CHEK2 kinase activity, results in approximately twofold increase of breast cancer risk in women and a tenfold increase of risk in men [15]. Somatic CHEK2 loss of heterozygosity is present in sporadic such as familial breast cancer suggesting this gene to be a classic suppressor gene. In our dataset, 50% of samples CHEK2 results deleted, while in the female dataset there is a 37% of deletion and 25% of amplifications. Other genes, such as KLF family member, seem to play a critical role in the growth and metastasis of numerous tumor types, at least in part by regulating the expression of cell-cycle genes, but they should be further investigated [3].

The present data confirmed the polygenic model of hereditary breast cancer susceptibility, stressing the role of particular genes in MBC. The concordance between the results obtained and the expectation as most

of the over-expressed and under-expressed genes mapping at amplified and deleted chromosomal locations were not random and could have a direct implication on the tumor progression process.

Alterations in genes involved in regulation of mitosis and DNA repair mechanisms may lead to a situation of so-called genomic instability, in which the integrity of the genome is at risk. The analysis of DNA CNAs may result in an appropriate strategy to understand tumor pathogenesis as well as to identify putative candidate genes to be used as diagnostic/prognostic markers or therapeutic targets of the disease.

References

- [1] B.A. Agnarsson, J.G. Jonasson, I.B. Bjornsdottir, R.B. Barkardottir, V. Egilsson and H. Sigurdsson, Inherited BRCA2 mutation associated with high grade breast cancer, *Breast Cancer Res. Treat.* **47** (1998), 121–127.
- [2] F. Andre, B. Job, P. Dessen, A. Tordai, S. Michiels, C. Liedtke, C. Richon, K. Yan, B. Wang, G. Vassal, S. Delaloge, G.N. Hortobagyi, W.F. Symmans, V. Lazar and L. Pusztai, Molecular characterization of breast cancer with high-resolution oligonucleotide comparative genomic hybridization array, *Clin. Cancer Res.* **15** (2009), 441–451.
- [3] C. Bureau, N. Hanoun, J. Torrisani, J.P. Vinel, L. Buscail and P. Cordelier, Expression and function of Kruppel like-factors (KLF) in carcinogenesis, *Curr. Genomics* **10** (2009), 353–360.
- [4] M.A. Caligo, S. Agata, G. Aceto, R. Crucianelli, S. Manoukian, B. Peissel et al., The CHEK2 c.1100delC mutation plays an irrelevant role in breast cancer predisposition in Italy, *Hum. Mutat.* **24** (2004), 100–101.
- [5] K.B. Contractor, K. Kaur, G.S. Rodrigues, D.M. Kulkarni and H. Singhal, Male breast cancer: is the scenario changing, *World J. Surg. Oncol.* **6** (2008), 58–69.

- [6] S.J. Diskin, T. Eck, J. Greshock, Y.P. Mosse, T. Naylor, C.J. Stoeckert Jr. et al., STAC: A method for testing the significance of DNA copy number aberrations across multiple array-CGH experiments, *Genome Res.* **16** (2006), 1149–1158.
- [7] S.L. Dombernowsky, M. Weischer, K.H. Allin, S.E. Bojesen, A. Tybjaerg-Hansen and B.G. Nordestgaard, Risk of cancer by ATM missense mutations in the general population, *J. Clin. Oncol.* **26** (2008), 3057–3062.
- [8] M. Falchetti, R. Lupi, P. Rizzolo, K. Ceccarelli, I. Zanna, V. Calò et al., BRCA1/BRCA2 rearrangements and CHEK2 common mutations are infrequent in Italian male breast cancer cases, *Breast Cancer Res. Treat.* **110** (2008), 161–167.
- [9] I.S. Fentiman, A. Fourquet and G.N. Hortobagyi, Male breast cancer, *Lancet* **367** (2006), 595–604.
- [10] D. Ford, D.F. Easton, M. Stratton, S. Narod, D. Goldgar, P. Devilee et al., Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium, *Am. J. Hum. Genet.* **62** (1998), 676–689.
- [11] L.S. Friedman, S.A. Gayther, T. Kurosaki, D. Gordon, B. Noble, G. Casey et al., Mutation analysis of BRCA1 and BRCA2 in a male breast cancer population, *Am. J. Hum. Genet.* **60** (1997), 313–319.
- [12] M.F. Ismail, M.S. Aly, H.M. Khaled and H.M. Mohamed, Detection of HER-2/neu, c-myc amplification and p53 inactivation by FISH in Egyptian patients with breast cancer, *Ger. Med. Sci.* **7** (2009), Doc03.
- [13] A. Mangia, P. Chiarappa, S. Tommasi, A. Chiriatti, S. Petroni, F. Schittulli et al., Genetic heterogeneity by comparative genomic hybridization in BRCAx breast cancers, *Cancer Genet. Cytogenet.* **182** (2008), 75–83.
- [14] C. Martinez-Bouzas, E. Beristain, I. Guerra, J. Gorostiaga, J.L. Mendizabal, J.L. De-Pablo et al., CHEK2 1100delC is present in familial breast cancer cases of the Basque country, *Breast Cancer Res. Treat.* **103** (2007), 111–113.
- [15] H. Meijers-Heijboer, A. van den Ouweland, J. Klijn, M. Wasielewski, A. de Snoo, R. Oldenburg et al., Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in non-carriers of BRCA1 or BRCA2 mutations, *Nat. Genet.* **31** (2002), 55–59.
- [16] F. Menolascina, S. Tommasi, P. Chiarappa, V. Bevilacqua, G. Mastronardi and A. Paradiso, Data mining techniques in aCGH-based breast cancer subtype profiling. An immune perspective with comparative study, *BMC Syst. Biol.* **1** (2007), 70–71.
- [17] E.P. Ojopi, L.R. Cavalli, L.M. Cavaliere, J.A. Squire and S.R. Rogatto, Comparative genomic hybridization analysis of benign and invasive male breast neoplasms, *Cancer Genet. Cytogenet.* **134** (2002), 123–126.
- [18] A.B. Olshen, E.S. Venkatraman, R. Lucito and M. Wigler, Circular binary segmentation for the analysis of array-based DNA copy number data, *Biostatistics* **5** (2004), 557–572.
- [19] L. Ottini, G. Masala, C. D'Amico, B. Mancini, C. Saieva, G. Aceto et al., BRCA1 and BRCA2 mutation status and tumor characteristics in male breast cancer: a population-based study in Italy, *Cancer Res.* **63** (2003), 342–347.
- [20] D. Pinkel, R. Seagraves, D. Sudar, S. Clark, I. Poole, D. Kowbel et al., High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays, *Nat. Genet.* **20** (1998), 207–211.
- [21] C. Rudlowski, H.J. Schulten, M.M. Golas, B. Sander, R. Barwing, J.E. Palandt et al., Comparative genomic hybridization analysis on male breast cancer, *Int. J. Cancer* **118** (2006), 2455–2460.
- [22] S.P. Shah, R.D. Morin, J. Khattra, L. Prentice, T. Pugh, A. Burleigh, A. Delaney, K. Gelmon, R. Guliany, J. Senz, C. Steidl, R.A. Holt, S. Jones, M. Sun, G. Leung, R. Moore, T. Severson, G.A. Taylor, A.E. Teschendorff, K. Tse, G. Turashvili, R. Varhol, R.L. Warren, P. Watson, Y. Zhao, C. Caldas, D. Huntsman, M. Hirst, M.A. Marra and S. Aparicio, Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution, *Nature* **461** (2009), 809–813.
- [23] V. Silvestri, P. Rizzolo, I. Zanna, M. Falchetti, G. Masala, S. Bianchi et al., PALB2 mutations in male breast cancer: a population-based study in central Italy, *Breast Cancer Res. Treat.* **122** (2010), 299–301.
- [24] S. Solinas-Toldo, S. Lampel, S. Stilgenbauer, J. Nickolenko, A. Benner, H. Dohner et al., Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances, *Genes Chromosomes Cancer* **20** (1997), 399–407.
- [25] M. Tirkkonen, T. Kainu, N. Loman, O.T. Johannsson, H. Olsson, R.B. Barkardottir et al., Somatic genetic alterations in BRCA2-associated and sporadic male breast cancer, *Genes Chromosomes Cancer* **24** (1999), 56–61.
- [26] S. Tommasi, A. Crapolicchio, R. Lacalamita, M. Bruno, A. Monaco, S. Petroni et al., BRCA1 mutations and polymorphisms in a hospital-based consecutive series of breast cancer patients from Apulia, Italy, *Mutat. Res.* **578** (2005), 395–405.
- [27] S. Tommasi, B. Pilato, R. Pinto, A. Monaco, M. Bruno, M. Campana et al., Molecular and *in silico* analysis of BRCA1 and BRCA2 variants, *Mutat. Res.* **644** (2008), 64–70.
- [28] J.R. Weiss, K.B. Moysich and H. Swede, Epidemiology of male breast cancer, *Cancer Epidemiol. Biomarkers Prev.* **14** (2005), 20–26.
- [29] L.H. Williams, D. Choong, S.A. Johnson, I.G. Campbell, Genetic and epigenetic analysis of CHEK2 in sporadic breast, colon and ovarian cancers, *Clin. Cancer Res.* **12** (2006), 6967–6972.
- [30] M. Wolf, S. Mousses, S. Hautaniemi, R. Karhu, P. Huusko, M. Allinen et al., High-resolution analysis of gene copy number alterations in human prostate cancer using CGH on cDNA microarrays: impact of copy number on gene expression, *Neoplasia* **6** (2004), 240–247.
- [31] J. Yang, Y. Ren, L. Wang, B. Li, Y. Chen, W. Zhao et al., PTEN mutation spectrum in breast cancers and breast hyperplasia, *J. Cancer Res. Clin. Oncol.* **136** (2010), 1303–1311.
- [32] W. Yu, Y. Kanaan, Y.K. Bae and E. Gabrielson, Chromosomal changes in aggressive breast cancers with basal-like features, *Cancer Genet. Cytogenet.* **193** (2009), 29–37.