2. CYTOGENETIC CHARACTERIZATION OF CONTROL CELL LINES

2.1 INTRODUCTION

The MCF7, T47D, BT474 and SKBR3 breast cancer cell lines are commonly used in experimental studies of cellular function, and much of the current knowledge of molecular alterations in breast cancer has been obtained from these cell lines (12, 106-108).

Whole-genome studies using microarray expression analyses have identified distinct subtypes of breast carcinomas (luminal, HER2+, and basal-like subtypes) based on the expression of approximately 500 genes (the so-called "intrinsic gene list") (109-111). These molecular subtypes have been approximated using immunohistochemical markers. In this way, estrogen (ER) and progesterone receptor (PR)+/*HER2*- tumors are classified as belonging to the luminal A molecular subtype, ER+/PR+/*HER2*+ tumors to the luminal B subtype, ER-/PR-/*HER2*+ tumors to the *HER2* subtype, and triple negative (ER-/PR-/*HER2*-) tumors to the basal-like carcinomas (112).

As determined by immunohistochemistry, the receptor profile classifies MCF7 and T47D cells (ER+/PR+/HER2-) as belonging to the luminal A subtype, BT474 cells (ER+/PR+/HER2+) as luminal B and SKBR3 cells (ER-/HER2+) as HER2 (113, 114). However, the mRNA transcriptional profile determined by whole genome oligonucleotide microarrays (106, 108, 115) allowed the characterization of all four-cell lines as luminal, because both ERα-regulated genes (e.g., MYB, RET, EGR3, and TFF1) (106) and genes associated with luminal epithelial differentiation (e.g., GATA3 and FOXA1) was expressed.

Different works have assayed the DNA genetic profile of these cell lines using comparative genomic hybridization (CGH) and multiplex ligation-dependent probe amplification (MLPA) to describe many different copy number alterations (115-117). However, these techniques are not able to detect either balanced chromosome rearrangements (e.g., translocations or inversions) or low frequency mosaicisms (< 30% abnormal cells). These chromosomal alterations may be assessed on metaphases using G-banding karyotype and multicolor fluorescence *in situ* hybridization (M-FISH) (12, 116-120). However, both procedures are time consuming and have been applied to only a small number of metaphases (12, 116-121). Thus, a search for clonal chromosomal aberrations within each cell line (12, 116-120) and a comprehensive comparison of the MCF7, T47D, BT474 and SKBR3 cell lines from a cytogenetic perspective have not yet been performed.

In the present chapter, structural and numerical alterations of MCF7, T47D, BT474 and SKBR3 breast cancer cell lines using a combination of G-banding and M-FISH

were determined. This has allowed us to determine the presence of cell clonality within each cell line and to thoroughly compare the cytogenetic components of the cell lines by clustering analysis.

2.2 METHODS

2.2.1 Cell lines

The human breast cancer cell lines MCF7 (ER+/HER2-), T47D (ER+/HER2-), BT474 (ER+/HER2+) and SKBR3 (ER-/HER2+) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) (121) in March 2010. Short tandem repeat (STR) analyses are routinely performed by ATCC during both accession and culture replenishment to avoid distributing misidentified cell lines to the scientific community. When received by our lab, these cell lines were expanded, and 3 vials were immediately frozen; cells from these stocks were later used for the experiments. These cell lines were analyzed by quantitative PCR (qPCR) and immunohistochemical tests in order to evaluate the gene expression of epithelial markers (keratins 8 and 18) and the presence of specific receptors (ERα, PGR, HER2, AR and EGFR). The protein expressions of ERα and HER2 were also confirmed by western blot.

The cell lines MCF7, T47D, and SKBR3 were maintained in a RPMI 1640 medium (Sigma, St. Louis, MO, USA), while the BT474 cell line was grown in a DMEM medium (Sigma). The culture media was supplemented with 10% fetal bovine serum (FBS) (Sigma), antibiotic-antimycotic solution (1X) (Sigma) and L-glutamine (2 mM) (Invitrogen GmbH, Karlsruhe, Germany). All cell lines were grown in an incubator at 37ºC and 5% CO2 and were evaluated to be free of mycoplasma contamination by a PCR assay. Cell line characteristics and culture conditions are further described in supplemental information (Annex 1).

2.2.2 Metaphase spread and G-Banding

Metaphases were obtained using standardized harvesting protocols for conventional and molecular cytogenetic analysis (M-FISH). Briefly, a colcemid solution (0.03 µg/ml) (Sigma) was added to cultures 2.5 hours before cell harvesting; cells were then treated with hypotonic solution, fixed three times with Carnoy's fixative (3:1 methanol to acetic acid) and spread on glass.
Glass slides were baked at 70°C for 24 hours, incubated in HCl and placed in 2xSSC buffer before treatment with Wright's stain. Image acquisition and subsequent karyotyping of metaphases were performed using a Nikon microscope with the cytogenetic software CytoVision System (Applied Imaging, Santa Clara, CA, USA). Chromosomal aberrations were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013 (122).

2.2.3 Multi-color FISH (M-FISH)

M-FISH was performed with the aim of identifying complex chromosomal rearrangements. The probe cocktail containing 24 differently labeled chromosome-specific painting probes (24xCyte kit MetaSystems, Altlussheim, Germany) was denatured and hybridized to denatured tumor metaphase chromosomes according to the manufacturer's protocol for the Human Multicolor FISH kit (MetaSystems). Briefly, the slides were incubated at 70°C in a saline solution (2xSSC), denatured in NaOH, dehydrated in ethanol series, air-dried, covered with 10 µl of probe cocktail (denatured) and hybridized for two days at 37°C. The slides were then washed with post-hybridization buffers, dehydrated in ethanol series and counter-stained with 10 µl of DAPI/antifade. The signal detection and analysis of subsequent metaphases used the Metafer system and Metasytems' ISIS software (software for spectral karyotypes).

2.2.4 Hierarchical clustering

The first cluster analysis was performed to assess the chromosomal heterogeneity of each cell line by considering the type and frequency of chromosomal alterations within metaphases. Each alteration was computed as present or absent within the karyotype of different metaphases. In the second cluster analysis, the frequency (%) of each chromosomal alteration was compared among the four cell lines. Hierarchical clustering was performed using package gplots from the Bioconductor project (http://www.bioconductor.org) for the R statistical language (123). A Euclidean distance was used to calculate the matrix of distances, and clusters were built using Ward's method.

2.3 RESULTS

Between 19 and 26 metaphases with good chromosomal dispersion and morphology were analyzed for each cell line in order to identify the structural and numerical alterations, and 100 metaphases for each cell line were analyzed to determine the level of ploidy. The rate and type of chromosomal abnormalities for each cell line are shown in Figure 10.

2.3.1 Cytogenetic profile and cluster analysis of MCF7 cells

The cytogenetic analysis performed on 26 metaphases of MCF7 cells showed a modal number hypertriploid to hypotetraploid (4n+/−) (76 to 88 chromosomes). Each chromosome harbored either a numerical or structural aberration, which accounted for 58 different rearrangements (31 numerical and 27 structural). Numerical alterations were present in all chromosomes, among which the

polyploidy was observed in 2% of the metaphases cells. Numerical alterations were present in all chromosomes; losses were more frequent than gains (Figure 10). Chromosomes 18 and 20 were nullisomic in 11.5% and 30.7% of the cells, respectively. Additionally, structural aberrations such as translocations, duplications and deletions were found in all chromosomes except 4, 5, 13, 14 and 18.

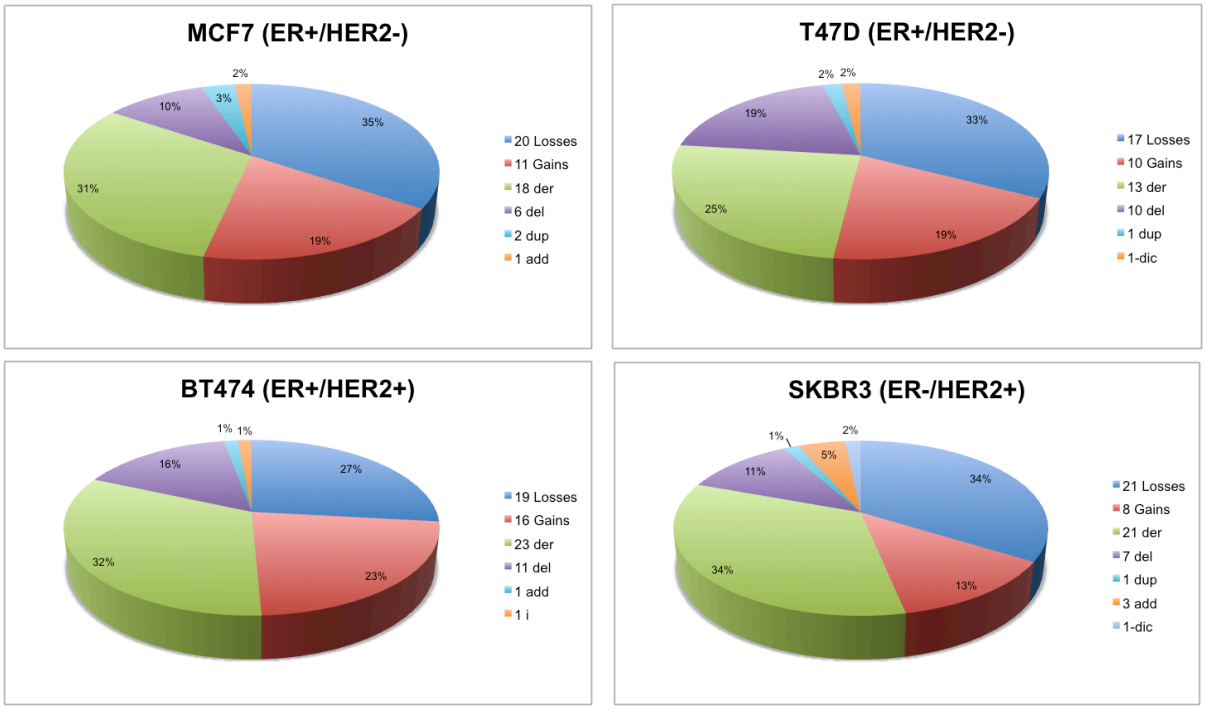

Figure 10. Distribution of numerical and structural aberrations for the four breast cancer cell lines. der= derivative chromosome; del= deletion; dup= duplication; add= additional material of unknown origin; dic= dicentric chromosome

The cluster analysis showed that these types of chromosomal alterations were similar for all 26 metaphases (horizontal dendrogram, Figure 11). Clustering by the frequency of the chromosomal aberration on the cell line resulted in 4 clusters (vertical dendrogram, Figure 11). The first cluster (red bar) represented chromosomal alterations that were frequently present; chromosome 7 was the most affected by structural abnormalities. The second cluster (blue bar) represents alterations that were present in all metaphases, including chromosomal losses and structural alterations of chromosomes 8 and 17.

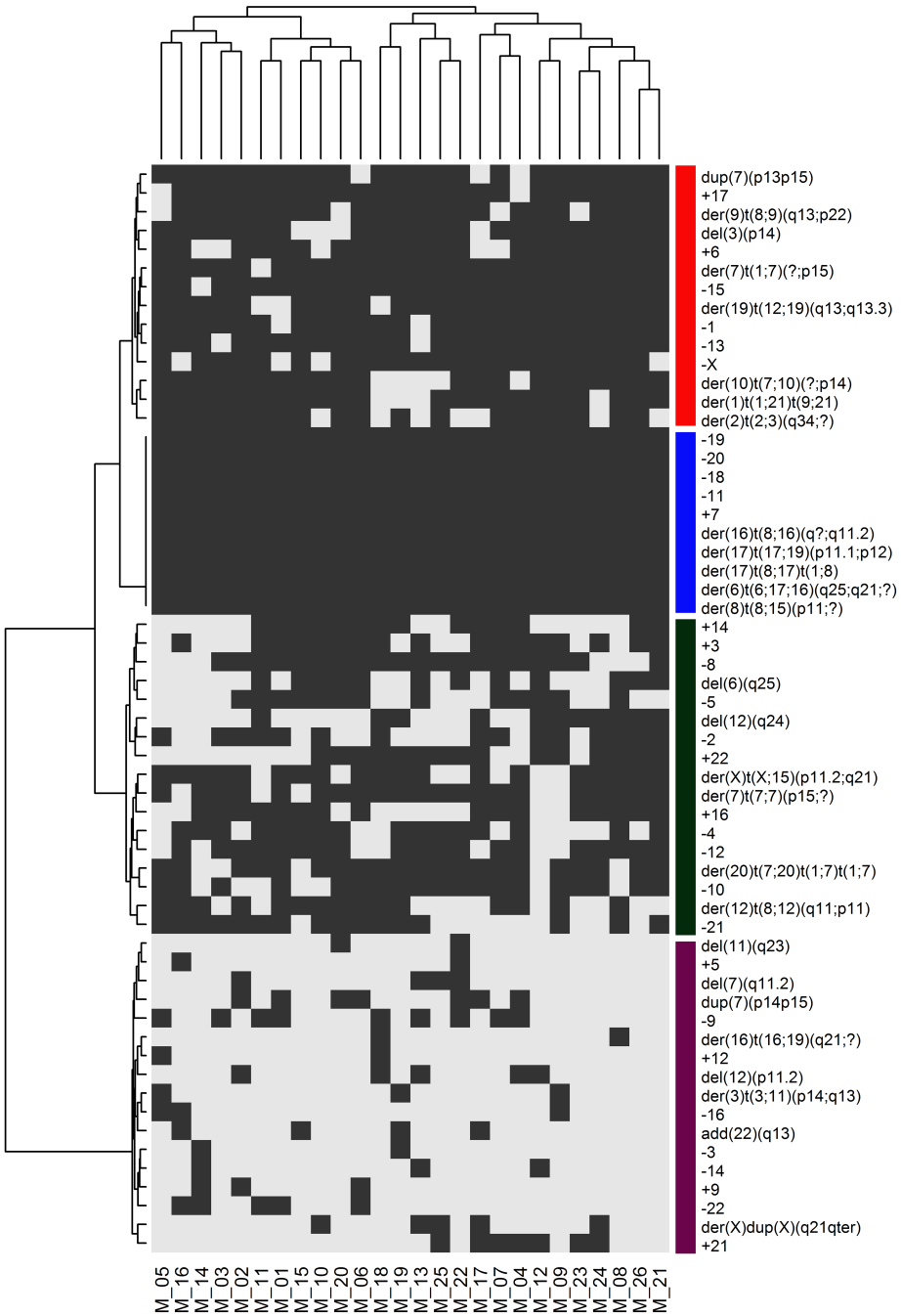

Figure 11. Hierarchical cluster analysis for presence or absence of chromosomal aberrations observed in 26 MCF7 metaphases. Each column refers to a metaphase (M) and each row to a chromosomal abnormality. Grey indicates presence and white indicates absence of each abnormality. Cluster number is indicated by vertical color bars. Cluster 1: red bar, cluster 2: blue bar, cluster 3: green bar and cluster 4: purple bar.

In particular, the loss of chromosomes 11, 18, 19 and 20 and gain of chromosomes 7 and 17 were observed in all metaphases. der(6)t(6;17;16)(q25;q21;?),der(8)t(8;15)(p11;?),der(16)t(8;16)(q?;q11.2),der(17)t(8;17)t(1;8) and der(17)t(17;19)(p11.1;p12) were present in all cells as a consequence of structural aberrations (Table 8 and Figures 12A and 12B).

Table 8. G-Banding and M-FISH karyotypes of all breast cancer cell lines studied.

Cell line	Karyotype
MCF7	76~88<4n>,-X[11],-Xx2[8],-Xx3[4],der(X)t(X;15)(p11.2;q21)[16], der(X)t(X;15)(p11.2;q21)x2[3],der(X)dup(X)(q21qter)[5],-1[22]-1x2[2], der(1)t(1;21)t(9;21)[22],-2[13],-2x2[2],der(2)t(2;3)(q34;?)[19],-3[2], +3[17],del(3)(p14)[22],der(3)t(3;11)(p14;q13)[3],-4[12],-4x2[4], +5[2],-5[13],+6[9],+6x2[8],+6x3[4],add(6)(q27)[2],del(6)(q25)[4], del(6)(q25)x2[8], der(6)t(6;17;16)(q25;q21;?)[26], +7[26],der(7)t(1;7)(?;p15)[23],der(7)t(1;7)(?;p15)x2[2], del(7)(q11.2)[4],dup(7)(p13p15)[7],dup(7)(p13p15)x2[5],dup(7)(p13p15)x3[11], dup(7)(p14p15)[5],dup(7)(p14p15)x2[2],der(7)t(7;7)(p15;?)[19],der(7)t(7;7)(p15;?)[2],-8[8], -8x2[12],der(8)t(8;15)(p11;?)[26],+9[3] -9[7],-9x2[2],der(9)t(8;9)(q13;p22)[22],-10[6],-10x2[10],-10x3[3], der(10)t(7;10)(?;p14)[9],der(10)t(7;10)(?;p14)x2[12],-11[14], -11x2[12],del(11)(q23)[2],-12[15],-12x2[4],+12[2], del(12)(p11.2)(5),del(12)(q24)[11],der(12)t(8,12)(q11;p11)[15], -13[12],-13x2[10],-13x3[2],-14[3],+14[14],-15[12],-15x2[10], -15x3[3],-16[3],+16[16],der(16)t(8;16)(q?;q11.2)[8],der(16)t(8;16)(q?;q11.2) x2[17]der(16)t(16;19)(q21;?)[2],+17[11],+17x2[10],+17x3[5],der(17)t(8;17)t(1;8)[21], der(17)t(8;17)t(1;8)x2[5],der(17)t(17;19)(p11.1;p12)x2[17],-18[4], -18x2[14],-18x3[5],-18x4[3],-19[7],-19x2[15],-19x3[4], der(19)t(12;19)(q13;p13.3)[21],der(19)t(12;19)(q13;p13.3)x2[2],-20[2], -20x2[5],-20x3[11],-20x4[8],der(20)t(7;20)t(1;7)t(1;7)[21],+21[5],+21x2[2],-21[14], -21x2[2],+22[12],+22x2[3],-22[3],-22x2[2],add(22)(q13)[4][cp26]
T47D	57~66<3n>,X,-X[24],der(X)t(X;6)(q12;p11)[24],-1[19],-2[22], -3[5],del(3)(p11)[2],del(3)(p14)[2],del(3)(p21)[2],del(3)(q13)[6],del(3)(q22)[3], der(3)ins(3;5)(p14;q13q31)[2],der(3)del(3)(p13)del(3)(q13q25)ins(3;5)(q13;q13q31)[2], -4[19],-5[2],+5[3],-6[17],+7[3],del(7)(p21)[3],del(7)(p13p14)[5], del(7)(p13p14)x2[10],del(7)(p13p15)[8],der(7)t(7;15)(q21;q13)[3],dup(7)(p13p14)[2],+8[12], der(8;14)(q10;q10)x2[24],-9[11],-9x2[9],-10[11],-10x2[10],del(10)(p10)[3], der(10)t(3;10)(q?;q24)del(10)(p11.2)[14],der(10)t(3;10)(q?;q24)del(10)(p11.2)x2[10],+11[9], +11x2[7],+11x3[2],der(11)t(11;17)(q23;q?)t(9;17)(q?12;?)[2],-12[2],+12[6],+12x2[4], del(12)(p12)[6],del(12)(q24.1)[5],del(12)(q24.1)x2[3],der(12)del(12)(p12)del(12)(q24)[4], der(12)t(12;13)(p12;q22)[10],der(12)t(12;16)(p11.2;?)[11],-13[16],-13x2[4],+14[3],+14x2[13], +14x3[3],-15[6],-15x2[18],-16[2],der(16)t(1;16)(q12;q12)dup(1)(q21q43)[24], dic(9;17)t(9;17)(p12;p13)[13],dic(9;17)t(9;17)(p12;p13)x2[11],-18[17],-18x2[4],-19[18], +20[9],+20x2[3],der(20)t(10;20)(q21;q13.3)[15],der(20)t(10;20)(q21;q13.3)x2[9], der(20)del(20)(p11)t(10;20)(q21;q13.3)[10],+21[10],+21x2[6],-21[2],-22[14][cp24]

BT474	65~106<4n>,X,-X[9],-Xx2[5],-Xx3[4],der(X)t(X;17)(q13;q11q12)del(X)(p21)[9], der(X)t(X;18;X;12)[2],del(X)(q22)[14],-1[6],-1x2[2],+1[3],del(1)(p36.1)[6], -2[7],+2[7],der(2)t(1;2;7;20)(?;q31;?;?)[18],+3[12],-3[3],del(3)(p11.2)[7], del(3)(p14)[2],del(3)(q11.2)[6],del(3)(q11.2)x2[8],del(3)(q21)[4],del(3)(q13)[2], -4[8],-4x2[9],+4[2],-5[9],-5x2[9],+6[11],+6x3[3],-6[3], del(6)(q13)[3],del(6)(q21)[3],der(6)t(6;7)(q25;q31)[7],der(6)t(6;7)(q25;q31)x2[16],+7[4], +7x2[6],+7x3[9],+7x4[3],der(7)t(7;20)(p13;?)[5], der(7)t(1;7)(?;q11.2)[9], del(7)(q11.2)[7],del(7)(q11.2)x2[3],del(7)(q11.2)x3[3],der(7)t(7;14)(p13;p11.2)[4],-8[10], -9[7],-9x2[4],-9x3[2],der(9)t(3;9)(q33;?)[3],+10[6],-10[5], der(10)t(10;16;19)(q25;?;?)[11],i(10)(q10)[4],+11[9],+11x2[2],-11[3], der(11)t(8;11)(q21.1;p15)[2],der(11)t(8;17)(q21.1;q11q12)t(11;17)(p15;q11q12)[8], der(11)t(8;17)(q21.1;q11q12)t(11;17)(p15;q11q12)x2[12],der(11)t(8;17)(q21.1;q11q12)t(11; 17)(p15;q11q12)x3[3],der(11)t(11;17)(q?14;?)t(8;17)(?;q?11.2)[13], der(11)t(11;17)(q?14;q?11.2)[9],+12[8], +12x2[5],del(12)(p11.1)[2],der(12)t(5;12)(q23;q23)[17],der(12)t(5;12)(q23;q23)x2[2],der(12) del(12)(p12)del(12)(q24)[3],-13[7],+13[6],+13x2[3],+13x4[2], der(13)t(13;17)(q10;q11q12)t(13;17)(q10;q11q12) [8],der(13)t(13;17)(q10;q11q12)t(13;17)(q10;q11q12)x2[12],+14[11], +14x2[3],+14x3[2],der(14)t(14;1;14)(q31;?;?)[6],der(14)t(14;1;14)(q31;?;?)x2[5], der(14)t(14;1;14)(q31;?;?)x3[9],der(14)t(14;1;14)(q31;?;?)x4[3], add(14)(p11.2)[2],der(14;14)(q10;q10)[3],der(14;14)(q10;q10)x2[16],-15[6],-15x2[9], -15x3[6],+16[7],+16x2[6],+16x3[3],-16[2],der(16)t(X;16)(q22;q24)[10], +17[16], der(17)t(6;17)(?;p13)t(15;17)(q11.2;q25)[22],-18[10],-18x2[4],-18x3[2],-19[6], -19x2[5],+19[5],-20[6],-20x2[6],+20[3],+20x3[2],der(20)t(19;20)(?;q10)[4], der(20)t(19;20)(?;q10)x2[5],+21[2],-21x2[11],-21x3[3],-22[2],-22x2[5],-22x3[2],-22x4[12], der(22)t(16;22)(q12;p11.2)[5][cp23]
SKBR3	76~83<4n>,XXX,-X[19],der(X)t(X;17)(q21;q?21)[15], der(X)t(X;8;17)(q13;q?21;?)[6],+1[8],+1x3[5],add(1)(p36.3)[4], del(1)(p13)[11],del(1)(p13)x2[6],del(1)(p34)[4],del(1)(p22)[9],del(1)(p36.1)[2], der(1)t(1;4)(q12;q12)[6],-2[6],-2x2[8],-2x3[3],der(2)t(2;6)(p13;?)[5],-3[10],-3x2[6],-4[8], -4x2[8],-4x3[3],der(4;14)t(4;14)(p11;p11.1)[3],-5[8], -5x2[8],-5x3[2],der(5)ins(5;15)(p13;q12q22)[6],-6[4],-6x2[12], -6x3[2],der(6)t(6;14;17)(q21;?;q11q12)del(6)(p23)[8],+7x2[8],+7x3[10], del(7)(q22)[12],del(7)(q32)[3],dup(7)(p14p15)[2],-8[6],+8[8], der(8)t(8;21)(?;?)t(8;21)(p23;?)t(8;21)(q24;?)[11],der(8)t(8;21)(?;?)t(8;21)(p23;?)t(8;21) (q24;?)x2[8],der(8)dup(8)(?)t(8;8)(?;p23)t(8;17)(q24;?)t(11;17)(?;?)[4], der(8;14)t(8;14)(p11.1;p11.1)[15],-9[9],-9x2[7],-10[4],-10x2[13],-10x3[2],+11[2],-11[7], add(11)(p15)[4],add(11)(q25)[2],-12[6],-12x2[5],+12[3],der(12)t(11;12)(p?;p12)[4], der(12)t(5;12)(q23;q23)[10],der(12)t(5;12)(q23;q23)x2[4],-13[6],-13x2[8], -13x3[3],der(13;13)(q11.2;q11.2)[16],-14[6],-14x2[4], der(14;14)(q11.2;q11.2)[18],-15[10],-15x2[7], dic(15;21)(p11.1;p11.1)[3], +16[4],-16[7],-17[3],+17[9],der(17;17)t(17;17)(q25;?)dup(17)(q22q25)t(17;20)(?;?)[5], der(17;17)t(17;17)(q25;?)dup(17)(q22q25)t(17;20)(?;?)x2[7], der(17;17)t(17;17)(q25;?)dup(17)(q22q25)t(17;20)(?;?)x3[7],del(17)(p11.2)[7], der(17)t(8;17)(q12;?)dup(17)(?)[19],der(17)t(8;17)(?;q25)dup(17) (q22q25)[5],der(17)t(8;17)(?;q25)dup(17)(q22q25)x2[2],der(17)t(8;13;14;17;21)(?;q?;q?;q11 q12;?)[8],der(17)t(3;8;13;17;20)(?;?;q12;?p;?)[12],der(17)t(3;8;13;17;20)(?;?;q12;?p;?)x2[2], -18[3],-18x2[11],-18x3[5],der(18)t(18;22)(p11.2;?)[12],-19[4],-19x2[7],-20[8],-20x2[4], -20x3[7],-21[6],-21x2[3],-22[9],-22x2[4],+22[2],der(22)t(19,22)(q?;q13)[5][cp19]

The number of metaphases analyzed is reported in brackets at the end of each karyotype. Also the frequency of each rearrangement identified is described in brackets.

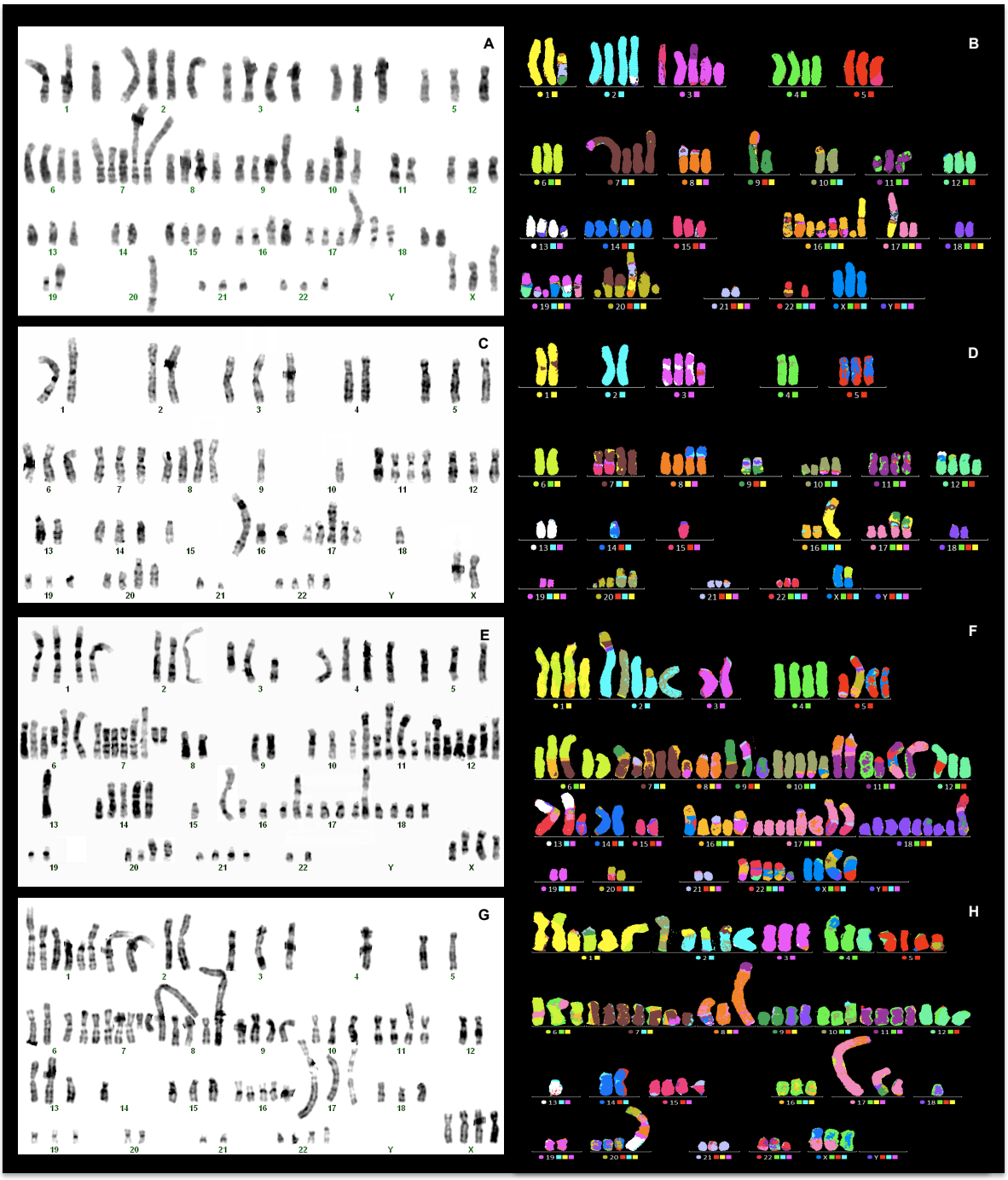

Figure 12. G-Banding and molecular cytogenetic results from four-breast cancer cell lines. A-B) G-banded and M-FISH karyotype of a representative metaphase of MCF7 cells. C-D) G-banded and M-FISH karyotype of a representative metaphase of T47D cells. E-F) G-banded and M-FISH karyotype of a representative metaphase of BT474 cells. G-H) G-banded and M-FISH karyotype of a representative metaphase of SKBR3 cells.

Less frequent alterations (mainly numerical) constituted cluster 3 (green bar), and very rare alterations (ranging from 0 in metaphases M_21 and M_26 to 5 in metaphases M_13 and M_22) constituted cluster 4 (purple bar).

2.3.2 Cytogenetic profile and cluster analysis of T47D cells

For the T47D cell line, 24 metaphases were examined. The modal number was near triploidy (3n+/−) (57 and 66 chromosomes). T47D cells had 52 different chromosomal alterations (27 numerical and 25 structural) (Figure 10). Furthermore, polyploidy was observed in 4% of the analyzed cells, and numerical chromosomal alterations were present in all chromosomes. In addition, structural aberrations (deletions, translocations, and duplications) were found in all chromosomes except 2, 4, 18, 19, 21 and 22. Similar to MCF7 cells, chromosomal alterations were almost homogeneously distributed among the 24 metaphases of T47D cells as demonstrated by hierarchical clustering (horizontal dendrogram, Figure 13).

When the frequency of chromosomal alterations was analyzed, 3 clusters were identified (see vertical dendrogram Figure 13), the first and largest cluster (red bar) was formed by common numerical alterations with a prevalence of losses. Additionally, rare structural aberrations were essentially observed in this cluster on chromosome 12. In the second cluster (the smallest, blue bar), der(X)t(X;6)(q12;p11), der(8;14)(q10;q10), der(10)t(3;10)(q?;q24)del(10)(p11.2), der(16)t(1;16)(q12;q12)dup(1)(q21q43), dic(9;17)t(9;17)(p12;p13) and der(20)t(10;20)(q21;q13.3) were present in all metaphases as the result of translocations, as well as the loss of chromosomes 15 and X (Table 8 and Figure 12C and 12D). The third cluster (green bar) has many rare abnormalities (ranging from zero in metaphases M_17 and M_21 to 4 in metaphases M_11 and M_10), most of which were structural (Figure 13).

2.3.3 Cytogenetic profile and cluster analysis of BT474 cells

For BT474 cells, 23 metaphases were examined. These cells showed the highest frequency of numerical and complex structural aberrations among all cell lines. These cells had a modal number near tetraploidy (4n+/−), which is between 65 to 106 chromosomes. Also they showed 35 numerical and 36 structural aberrations (Figure 10), whereas polyploidy was not present.
Similar to the previous cell lines, cluster analysis of BT474 cells showed nearly homogeneous chromosomal alterations in all metaphases (horizontal dendrogram, Figure 14). Also, in this case, isochromosomes, deletions and derivatives were frequently found (Table 8 and Figure 12E and 12F). Also, numerical alterations were observed on all chromosomes, with losses being more frequent than gains. Losses of chromosomes X, 15 and 22 were observed in 78%, 91% and 91% of metaphases, respectively, while the gain of chromosome 7 was identified in 96% of cells.

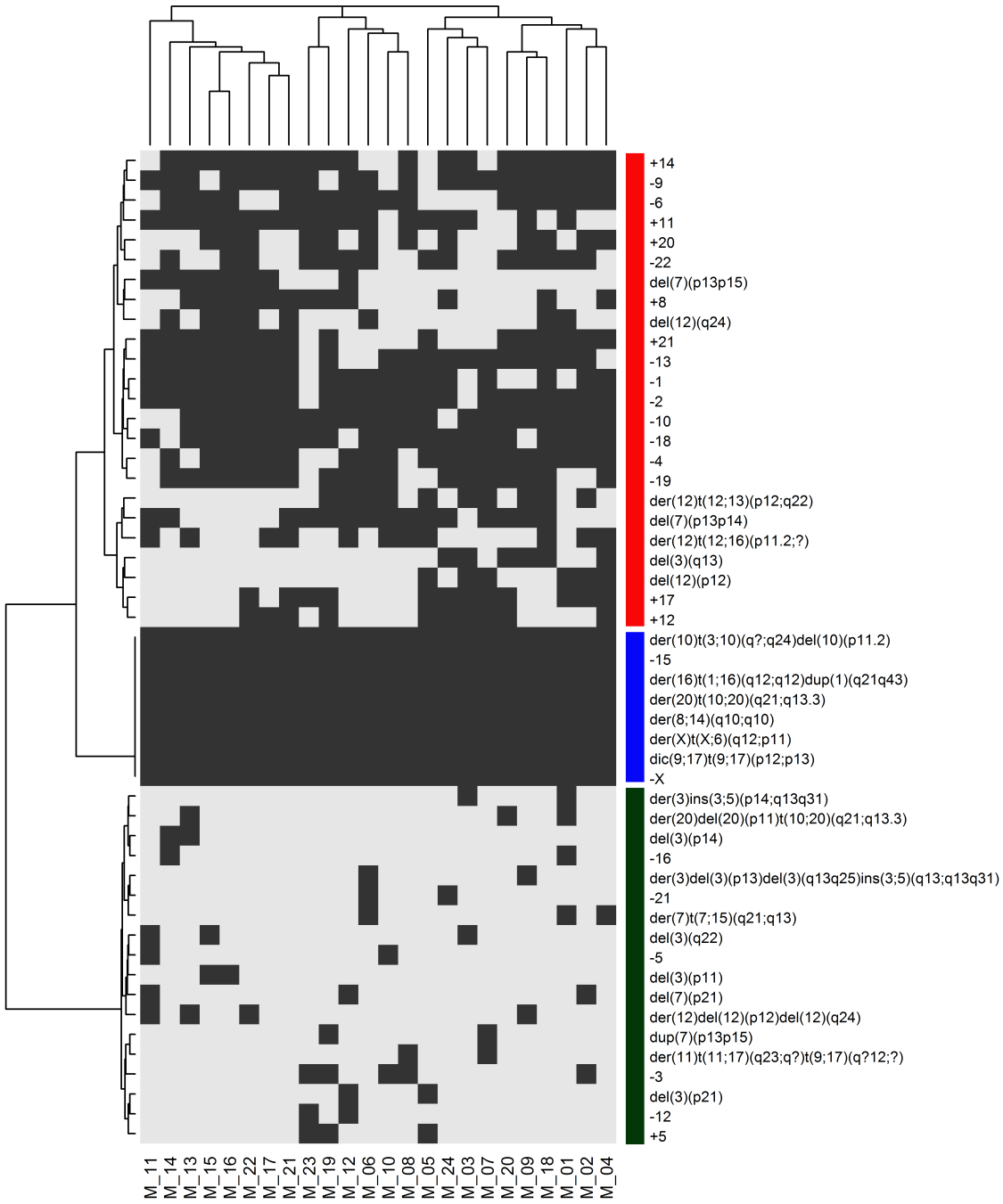

Figure 13. Hierarchical cluster analysis for presence or absence of chromosomal aberrations observed in 24 T47D metaphases. Each column refers to a metaphase (M) and each row to a chromosomal abnormality. Grey indicates presence and white indicates absence of each abnormality. Cluster number is indicated by vertical color bars. Cluster 1: red bar, cluster 2: blue bar and cluster 3: green bar.

The frequency analysis for alterations within the BT474 cell line resulted in 2 clusters (vertical dendrogram). The first cluster (red bar), presented both numerical and structural alterations in almost all cells. Only three structural alterations were observed among all metaphases. These were der(6)t(6;7)(q25;q31), der(11)t(8;17;11)(q21.1;?;p15) and der(14;1;14)(q31;?;?) (Table 8 and Figure 12E and 12F). The second cluster (blue bar) included sporadic aberrations with a minimum of 3 such alterations observed in metaphase M_22 (Figure 14).

2.3.4 Cytogenetic profile and cluster analysis of SKBR3 cells

In the SKBR3 cell line, 19 metaphases were examined. These cells showed a hypertriploid to hypotetraploid (4n+/−) karyotype (76 to 83 chromosomes). Polyploidy was observed in 19% of all cells. The SKBR3 cells had 29 numerical and 33 structural aberrations (Figure 10). Numerical alterations were observed in all chromosomes. Structural aberrations (translocations, deletions, and duplications) were found in all chromosomes except 3, 9, 10 and 16 (Table 8 and Figure 12G and 12H).

In comparison to other cell lines, hierarchical clustering showed similarities of chromosomal alterations among the 19 metaphases (horizontal dendrogram, Figure 15). Clustering by the frequency of chromosomal alterations defined 3 clusters (Figure 15). The first, and largest, cluster (red bar) was formed by sporadic aberrations, with structural aberrations being the most prevalent. The second cluster (blue bar) included frequent rearrangements, with more numerical than structural aberrations. The third and smallest group (green bar) contained chromosomal abnormalities that were present in all cells, both numerical, such as monosomies of chromosomes X, 4, 10, 18 and 20, and structural, such as those on chromosomes 1, 8 and 17.

2.3.5 Comparison of the four cell lines

Using hierarchical clustering, five major clusters were identified (Figure 16). One cluster was characterized mainly by numerical chromosomal abnormalities (18 losses and 7 gains), which were common to the four cell lines. Only two structural alterations, der(14;14)(q10;q10) and der(12)t(5;12)(q23;q23), were common to the HER2+ cells. However, the other clusters encompassed cell type-specific abnormalities, which were primarily structural (Figure 16). This analysis revealed great similarity between the T47D and BT474 cells and some similarity between these two cell lines and the SKBR3 cell line. MCF7 cells presented a chromosomal pattern that was markedly different from those of the other cell lines (Figure 17).

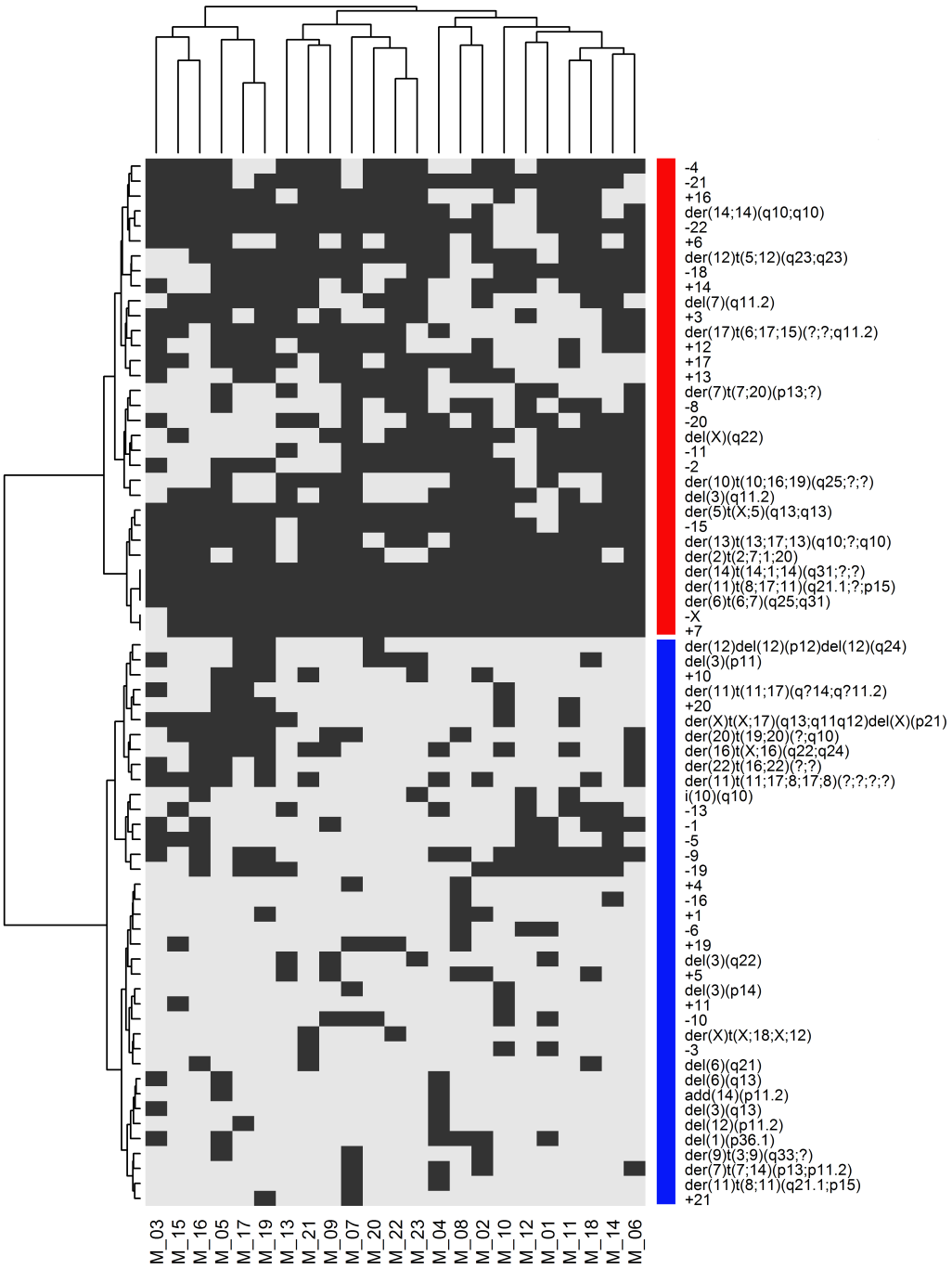

Figure 14. Hierarchical cluster analysis for presence or absence of chromosomal aberrations observed in 23 BT474 metaphases. Each column refers to a metaphase (M) and each row to a chromosomal abnormality. Grey indicates presence and white indicates absence of each abnormality. Cluster number is indicated by vertical color bars. Cluster 1: red bar and cluster 2: blue bar.

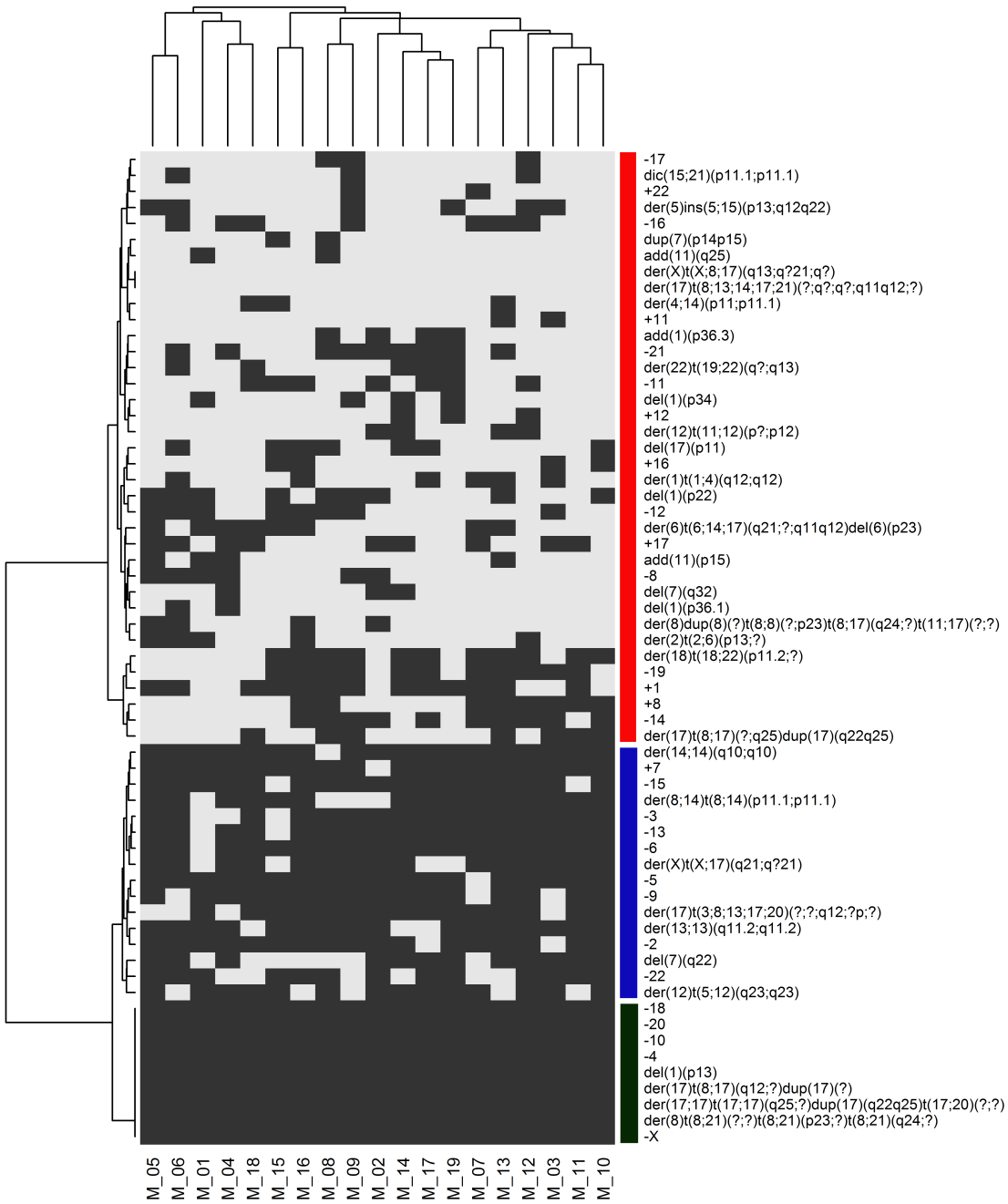

Figure 15. **Hierarchical cluster analysis for presence or absence of chromosomal aberrations observed in 19 SKBR3 metaphases**. Each column refers to a metaphase (M) and each row to a chromosomal abnormality. Grey indicates presence and white indicates absence of each abnormality. Cluster number is indicated by vertical color bars. Cluster 1: red bar, cluster 2: blue bar and cluster 3: green bar.

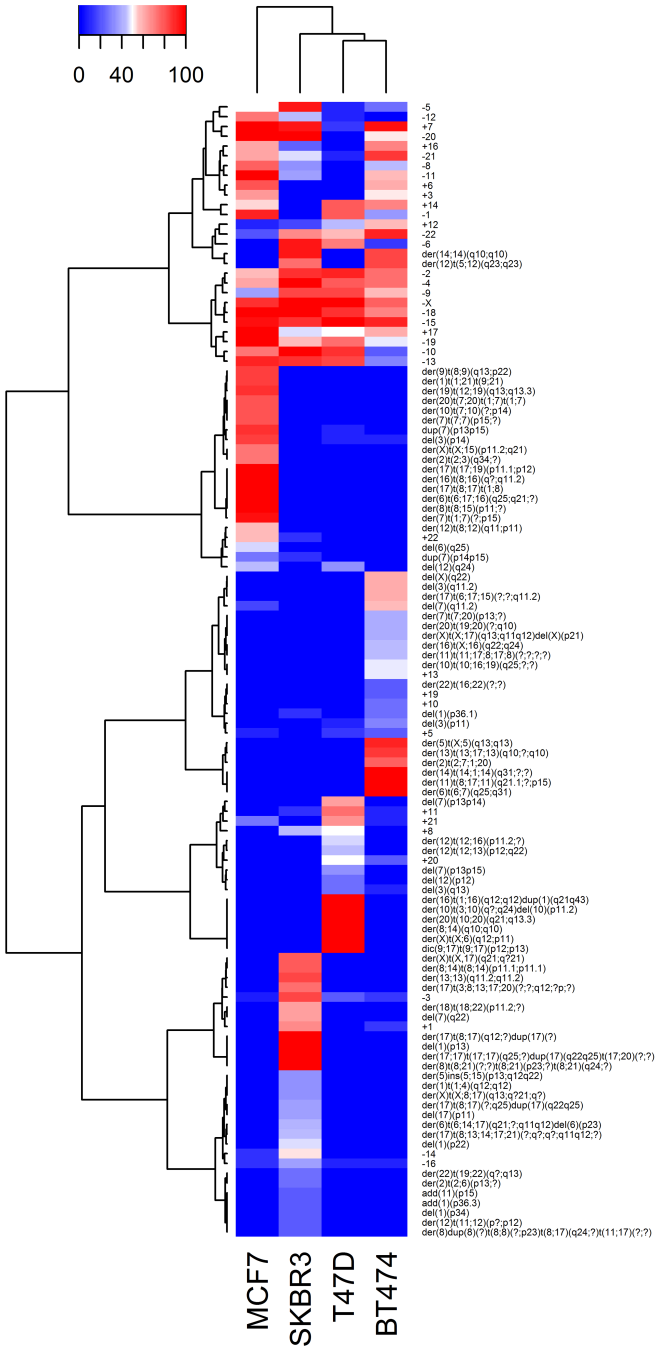

Figure 16. Hierarchical cluster analysis of percentage of chromosomal aberrations observed in four breast cancer cell lines. Clustering stratifies cell lines into five groups. The first cluster is characterized by the presence of numerical chromosomal abnormalities (aneuploidies) common to the four cell lines (ER+, ER-, HER2+, HER2-). The other clusters are comprised of chromosomal abnormalities specific to cell type. Gradient color indicates percentage of chromosomal abnormalities present in each cell line.

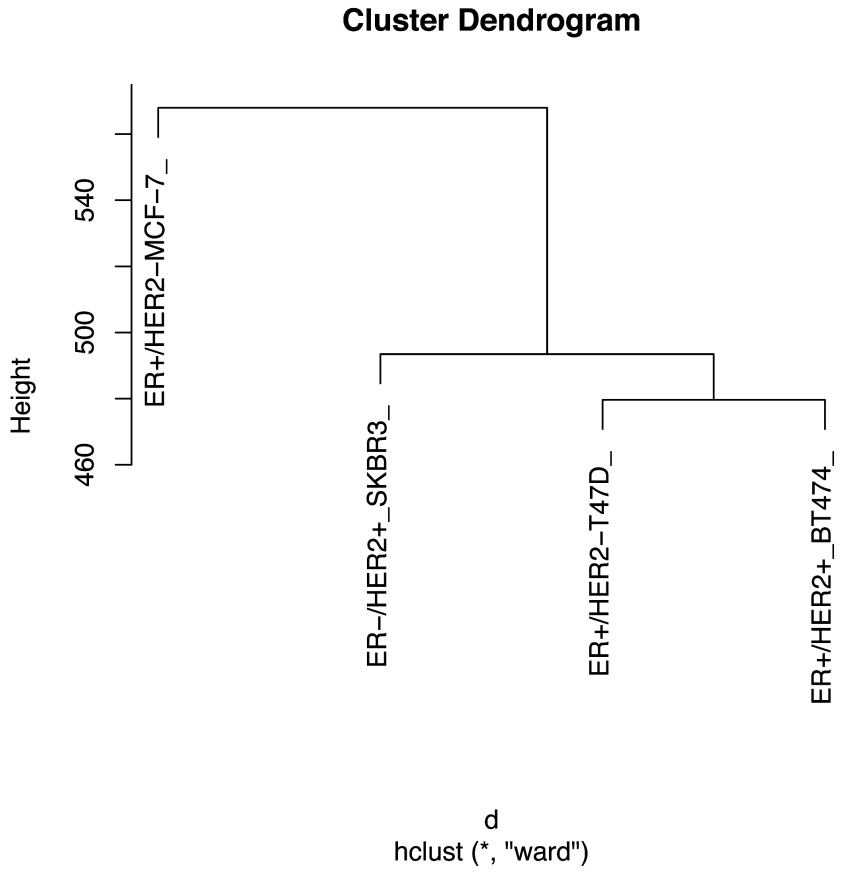

Figure 17. **Cluster dendogram from cytogenetic analysis of the four breast cancer cell lines.** The analyses confirm the higher similarities between the T47D and BT474 cells, and between these two cell lines and the SKBR3 cell line. The MCF7 cells show a chromosomal pattern markedly different from previous cells.

2.4 DISCUSSION

The MCF7 (ER+/HER2-), T47D (ER+/HER2-), BT474 (ER+/HER2+) and SKBR3 (ER-/HER2+) cell lines are widely used in breast cancer research as a model of the luminal and HER2 immunophenotypes (113, 114). Although, classical cytogenetic analysis is a time consuming method and lacks the resolution of molecular techniques, it is the best tool for obtaining an overall picture of the types and frequency of chromosomal changes. The results from G-Banding and M-FISH tests on a large number of metaphases allowed us to acquire a thorough insight into the type and frequency of chromosomal alterations in the MCF7, T47D, BT474 and SKBR3 cell lines and to detect previously unreported chromosome alterations (Table 9).

Cluster analysis excluded the presence of cell clones within each cell line because the same abnormalities were homogenously observed in all metaphases. Conversely, within the same cell line, the frequency of each chromosomal alteration was variable and defined different clusters. Finally, the comparative cluster analysis of these four cell lines showed that they shared up to 5 numerical aberrations in more than 50% of the metaphases (-2, -4, -15, -18, -X) and that the chromosomal structural alterations were cell-type specific, with the exception of two derivative chromosomes that were shared by the BT474 and SKBR3 HER2+ cell lines.

The HER2+ cell lines, BT474 and SKBR3, showed the highest frequency of numerical and structural aberrations when compared with the HER2- cell lines MCF7 and T47D. Polyploidy, which was more frequent in HER2+ than HER2- cells, has been correlated with short survival, drug resistance and metastasis (124). In addition, complex chromosomal alterations affecting chromosomes 8, 11, and 17 were frequently observed in HER2+ cells. These chromosomes contain genes that are commonly involved in the invasion, metastasis and pathogenesis of breast cancer, such as *c-MYC* on 8q24; *HRAS, CD151, CTSD* on 11p15; *CCND1* on 11q13 (125-129); and *TOP2A* on 17q21. Moreover, rearrangements of chromosome 17 were more frequent than its polysomy for HER2+ cells and carcinomas. Pathologists must consider this observation when diagnosing the HER2 amplification in interphase nuclei of breast carcinomas, which uses a ratio between HER2 copies and chromosome 17 centromere signals (102, 130).

On the other hand, among ER+ cells, MCF7 cells are cytogenetically different than both T47D (ER+/HER2-) and BT474 (ER+/HER2+) cells and are characterized by a specific subset of complex structural alterations, which are listed in the cluster analysis comparison of the four cell lines (Figure 15). In particular, chromosome 7 was frequently structurally and numerically affected, and polysomy of chromosome 7 was observed in all metaphases. This finding has been closely associated with lymph node metastasis and prognosis in breast cancer patients (131). One may speculate that the differences observed in the pattern of chromosomal aberrations between the MCF7 and T47D cell lines could partly explain the differences in the profiles of protein expression that were recently identified in these cells (132). Proteomic studies have revealed that a high number (at least 164) of proteins (including proteins involved in the regulation of breast cancer cell growth) are expressed differentially by T47D and MCF7 cells (132). For example, of the proteins that are principally involved in cell proliferation and apoptosis and are upregulated in MCF7 cells, the Chromobox protein homolog 3 and the Cytochrome c-releasing factor 21 are encoded by genes mapped to chromosome 7, which was typically polysomic in MCF7 cells, as reported above.

Table 9. Comparison of selected chromosomal aberrations detected in MCF7, T47D, BT474 and SKBR3 cell lines in previous studies with our G-banding and M-FISH results

Cell line	ATCC(121)	National Center for Biotechnology Formation NCBI(133)	Gasparini, et al. 2010(119)	Davidson, et al. 2000(118)	G-banding and M-FISH present study
MCF7	NR	NR	dup(X)(?;qter)	der(1)t(X;1)	der(X)dup(X)(q21qter)
	NR	NR	NR	NR	der(6)t(6;17;16)(q25;q21;?)
	NR	der(17)t(17;20)(q25;?)t(1;20)t(1;3or7)	NR	der(?)t(11;1;17;19;17)	der(17)t(17;19)(p11.1;p12)
	NR	NR	NR	der(?)t(17;1;19;17;20)	der(17)t(8;17)t(1;8)
T47D	der(8)t(8;14)	der(8)t(8;14)(p21;q21)	_	der(8)t(8;14)	der(8;14)(q10;q10)
	der(9)t(9;17)	der(9)t(9;17)(p12;q?11)	_	NR	dic(9;17)t(9;17)(p12;p13)
	der(10)t(10;20)	der(20)t(10;20)(q21;q13)	_	NR	der(20)t(10;20)(q21;q13.3)
BT474	der(6)t(6;7)(q21;q21)	_	der(6)t(6;7)(q25;?)	_	der(6)t(6;7)(q25;q31)
	NR	_	der(11)t(8;11;??)(?;p15;?)	_	der(11)t(8;17;11)(q21.1;?;p15)
	NR	_	NR	_	der(11)t(11;17)(q?14;q?11.2)
	i(13q)	_	der(13;13)(q10;q10)	_	der(13)t(13;17;13)(q10;?;q10)
	der(14)t(14;?)(q32,?)	_	der(14)t(1;14;X)(?;q31;?)	_	der(14)t(14;1;14)(q31;?;?)
SKBR3	NR	_	NR	der(8)t(8;21)	der(8)t(8;21)(?;?)t(8;21)(p23;?)t(8;21)(q24;?)
	NR	_	NR	NR	der(8)dup(8)(?)t(8;8)(?;p23)t(8;17)(q24;?)t(11;17)(?;?)
	NR	_	NR	der(?)t(8;14)	der(8;14)t(8;14)(p11.1;p11.1)
	NR	_	NR	NR	der(17)t(8;17)(q12;?)dup(17)(?)
	NR	_	NR	der(?)t(20;19;8;17)	der(17;17)t(17;17)(q25;?)dup(17)(q22q25)t(17;20)(?;?)
	NR	_	NR	der(8?)t(13;3;8;3;8;13)	der(17)t(8;13;14;17;21)(?;q?;q?;q11q12;?)
	NR	_	NR	der(?)t(20;3;8;17;19;8;3;13)	der(17)t(3;8;13;17;20)(?;?;q12;?p;?)
	NR	_	NR	NR	der(17)t(8;17)(?;q25)dup(17)(q22q25)
	NR	_	NR	der(?)t(19;22)	der(22)t(19,22)(q?;q13)

Abbreviations: NR, not reported. Dashes indicate that no information was available

The karyotypic differences should be considered when experimental transfection studies are being designed, because it is possible that their complex chromosomal alterations may alter the results. MCF7 cells, which differ greatly from the BT474 and SKBR3 (HER2+) cells, are frequently used to study the effect of HER2 transfection (134-136); however, they may not represent the best substrate. Conversely, T47D cells (ER+/HER2-) and BT474 cells share some similarities in their chromosomal profiles, and also with SKBR3 cells. For example, T47D and BT474 cells share numerical alterations, such as losses of chromosome 6 and gains of chromosomes 11 and 20, but they have no structural abnormalities in common.

Aneuploidy has been described as one of the most common characteristics of the cancer genome (137). In addition, numerical abnormalities have been observed more frequently in primary cancers, while structural alterations and amplifications were more commonly observed in metastatic breast cancer (138). Given the above, one may hypothesize that the earliest genetic event may be aneuploidy, followed by structural alterations (138, 139). These structural alterations may lead to the deregulated expression of genes, such as a loss of tumor suppressor genes, the activation of oncogenes and the formation of fusion proteins with enhanced or aberrant transcriptional activity. For instance, some of the genes up regulated in HER2+ cell lines (140) reside on chromosomes 5, 6, 10, 19, and 20, which were reported to be polysomic in BT474 cells in the present study (Annex 2).

2.5 CONCLUSIONS

In conclusion, by using both conventional and molecular karyotyping, our work provides a comprehensive and specific characterization of complex chromosomal aberration profiles for MCF7, T47D, BT474 and SKBR3 cell lines, thus providing useful and important information for experimental studies. These cell lines serve as models for investigating the molecular biology of breast cancer; therefore, it may be essential to consider the potential influence of these chromosomal alterations when interpreting biological data.