

Variation between independently cultured strains of the MDA-MB-231 breast cancer cell line identified by multicolour fluorescence in situ hybridisation

Research Article

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Summary

Established cell lines derived from breast carcinomas provide important models, which can be used to study the genetics, biochemistry and dynamics of breast cancer *in vitro*. However, the very nature of these cell lines, along with their widespread and prolonged culture in plastic, may eventually lead to genotypic and phenotypic variations between strains cultured by different research groups. The aim of this study was to investigate the *in vitro* genetic divergence at the chromosome level of the breast cancer cell line MDA-MB-231. Multicolour fluorescence in situ hybridisation (MFISH) allows rapid detection, discrimination and karyotyping of all chromosomes within a single metaphase spread. Strains of MDA-MB-231 obtained from 3 different laboratories: (I) Hull, (II) York and (III) USA were subjected to MFISH analysis. Karyotypic aberrations were identified, which were common to all strains of this cell line, for example, the marker chromosomes der(2)t(2;12;8) and der(2)t(8;2). However, several other unique abnormalities were identified between strains enabling the production of a map of proposed karyotypic divergence. The potential genetic divergence of cell lines cultured extensively in the laboratory should therefore be taken into account during the interpretation of *in vitro* experimental data.

I. Introduction

Prior to new breast cancer therapies being entered into clinical trials they are thoroughly tested using strictly controllable model systems. Established breast cancer cell lines such as MCF-7, MDA-MB-231, T-47D and BT-20 are commonly utilised for this purpose and also as general *in vitro* models of breast cancer pathogenesis, progression, response to chemotherapy and drug resistance. In a recent review of the relevance of breast cancer cell lines as model systems, an extensive literature search revealed that studies involving MCF-7, MDA-MB-231 and T-47D accounted for over two thirds of all studies using breast cancer cell lines (Lacroix and Leclercq, 2004). Such cell lines retain many of the characteristics of their parent tumour (for example, the ER positive status of MCF-7) and are considered to represent the parent tumour both genotypically and phenotypically to a certain extent (remembering that tumours are by nature heterogeneous entities). Breast cancer cell lines are also

important tools in the field of drug discovery. Recently, they have been successfully used as a screening panel for the 4-aryloxy- and 4-arylsulfanyl-phenyl-2-aminothiazole compounds – a family of potential cancer cell growth inhibitors (Gorczyński et al, 2004). However, there are inherent problems with the use of cell lines in general and especially those derived from tumours. First, the phenomenon of intraspecies cross-contamination of established cell cultures appears to be widespread and may account for some misrepresentation of data (MacLeod et al, 1999). Second, the karyotypic evolution of the commonly used MCF-7 cell line in continued culture both over time, and between individual cultures grown at independent research facilities has been previously reported (Bahia et al, 2002).

We utilised multicolour fluorescence *in situ* hybridisation (MFISH) to analyse the karyotypic variation among independently cultured strains of another widely used breast cancer cell line - MDA-MB-231, to determine

whether this type of variation is limited to MCF-7, or is more widespread.

II. Materials and methods

A. Cell culture

Strains of MDA-MB-231 were obtained from the Department of Biology, University of York and Abbott Laboratories, Downers Grove, IL. Cells were cultured in RPMI growth media supplemented with 10% foetal calf serum, 1% glutamine, 1% penicillin streptomycin and 1% fungizone (all purchased from Invitrogen Ltd, Paisley, UK).

B. Preparation of metaphase spreads

Metaphase chromosome spreads were produced according to standard protocols (Ashman et al, 2002; Watson et al, 2004). Cultures at near confluence (60-70%) underwent an 18 hour incubation period with 10 µl of 10 µg/ml colcemid (final concentration 20 ng/ml). Cells were pelleted (200 g for 8 min), and the pellet resuspended in 8ml hypotonic solution (0.075M KCl). After incubation at 37°C for 20 minutes, cells were fixed in 3 changes of 3:1 methanol:acetic acid and stored for 18 to 24 hours at -20°C. Chromosome spreads were prepared by dropping 25 µl of suspension onto clean, humidified glass slides and allowed to air dry before visualisation using phase contrast microscopy (Nikon E800, Nikon UK Ltd, Kingston, England). Visualisation prior to the addition of MFISH probe allowed assessment of the quality of the metaphases produced as well as selection of the best quality spreads to be analysed.

C. MFISH

All MFISH reagents and analysis software systems were purchased from Abbott Laboratories (Maidenhead, UK). MFISH was carried out according to previously described protocols (Ashman et al, 2002; Watson et al, 2004). Slides were treated with pepsin and RNase to remove cytoplasmic protein and RNA, to maximise probe binding. The slides were fixed in 1% v/v formaldehyde and denatured in 70% formamide/2x salt sodium citrate (SSC) prior to the addition of 10 µl denatured SpectraVysion 24-colour probe and hybridization at 37°C for 72 hours. Post hybridization, slides were washed to remove non-

specifically bound probe and counterstained with 15 µl of 42 ng/ml DAPI in Antifade. Four to ten high power (100x objective) metaphase images were captured from each slide using a Nikon E800 epifluorescent microscope with a Ludl 6-position filter wheel (with filters for each of the six fluorochromes–SpectrumGold, SpectrumAqua, SpectrumRed, SpectrumGreen, SpectrumFRed and DAPI) and a Photometrics Sensys CCD camera. Images were analysed using Quips SpectraVysion analysis software on an Apple G3 Power Macintosh. All metaphase spreads identified prior to probe treatment were captured, however only those of sufficient quality were included in the final composite karyotype. Composite karyotypes were produced for each metaphase then compiled to give an overall karyotype for each strain (Table 1).

D. Analysis

As per ISCN guidelines (1995), abnormal chromosomes were included if two or more metaphase spreads exhibited the same aberration. Chromosomes were reported in shortened ISCN format as derivative (der) chromosomes.

III. Results

The composite karyotypes compiled from the raw MFISH data for each strain of the MDA-MB-231 cell line are displayed in Table 1. The chromosomal aberrations shared by each strain are highlighted in red, revealing many unique translocations and differences in chromosomal copy number. The chromosomal translocations shared by, and unique to, the three strains are further summarised in Table 2.

Figure 1 demonstrates the use of MFISH in the identification of derivative chromosomes and highlights two of the chromosomes common to all strains of the MDA-MB-231 cell line – der(2)t(2;12;8) and der(15)t(20;15). The derivative chromosome der(6)t(6;19;12;8), which is one marker that distinguishes the MDA-MB-231 (I) strain (Table 2), is also shown in Figure 1. A possible route for karyotypic divergence of the MBA-MB321 cell line is given in Figure 2

Table 1. Consensus karyotypes compiled using the MFISH data for each of the three MDA-MB231 stains (I), (II) and (III). The chromosome number range is given in bold at the beginning of each karyotype. Whole and derivative chromosomes are given as the modal number as derived from the compiled karyotypes of each strain. In some cases it was not possible to use the modal number. In accordance with ISCN guidelines (1995), in these instances each set of abnormalities were given along with the number of spreads containing the abnormality [n]. [cp n] identifies the number of individual spreads used to compile each composite karyotype. Chromosomal aberrations shared by each strain are highlighted in red.

Sample	Composite Karyotype
MDA-MB231 (I)	(38-99). 1x3, 2x1, der(2)t(2;12;8), der(2)t(8;2)x2, 3x1, der(3)t(3;8), 4x4[3], 4x3[3], 4x2[2] der(4)t(4;9), 5x2, der(5)t(5;7), 6x2, der(6), der(6)t(6;14), der(6)t(6;19;12;8), 7x2, der(8)t(18;8), 9x3, 10x3, 11x3, 12x2, 13x1, der(13)t(16;13), 14x2, der(14), der(11)t(11;15), der(15)t(20;15), 16x2, 17x3, 18x2, der(?)t(18;5), 19x2[4], 19x3[4], der(19)t(19;7), 20x2, 21x3, 22x1, Xx2 [cp 8].
MDA-MB231 (II)	(58-63). 1x3, 2x1, der(2)t(2;12;8), der(2)t(8;2)x2, 3x2, der(3)t(3;8), 4x3, 5x2, der(5)t(5;7), 6x2, der(6), der(6)t(6;14), der(6)t(6;19;12;8), 7x2, der(8)t(12;8), der(8)t(18;8), 10x3, 11x3, 12x2, 13x1, der(13)t(13;21), 14x3, der(11)t(11;15), der(15)t(20;15), 16x2, 17x3, 18x2, 19x2, der(19)t(19;7), 20x3, 21x1[2], 21x2[2], 21x3[2], Xx1, der(X)t(X;4) [cp 6].
MDA-MB231 (III)	(61-64). 1x3, 2x1, der(2)t(2;12;8), der(2)t(8;2)x2, 3x3, 4x3, 5x2, der(5)t(5;7), 6x2, der(6), der(6)t(6;14), 7x3, 8x1, der(8)t(12;8), der(8)t(18;8), 9x3, 10x3, 11x3, 12x2, 13x2, 14x2, der(14), der(11)t(11;15), der(15)t(20;15), 16x2, 17x3, 18x2, der(19)t(19;X;5), 19x2, 20x3, 21x1, der(21)t(21;8), 22x2, Xx2, der(X)t(5;X) [cp8].

Key: der = derivative chromosome, iso = isochromosome, dic = dicentric chromosome

Table 2. Derivative chromosomes identified by MFISH analysis of the three strains of MDA-MB231. Chromosomes identified in any one strain were only counted if found in two or more metaphase spreads (+ translocation present; - translocation absent). Translocations highlighted in red were shared by each of the three strains.

Translocation	MDA-MB231 (I)	MDA-MB231 (II)	MDA-MB231 (III)
der(2)t(8;2)	+	+	+
der(2)t(2;12;8)	+	+	+
der(3)t(3;8)	+	+	-
der(4)t(4;9)	+	-	-
der(5)t(5;7)	+	+	+
der6	+	+	+
der(6)t(6;14)	+	+	+
der(6)t(6;19;12;8)	+	-	-
der(8)t(8;18)	+	+	+
der(8)t(12;8)	-	+	+
der(13)t(13;21)	-	+	-
der(13)t(16;13)	+	-	-
der14	+	-	-
der(15)t(20;15)	+	+	+
der(15)t(11;15)	+	+	+
der(19)t(19;7)	+	+	-
der(19)t(19;X;5)	-	-	+
der(21)t(21;8)	-	-	+
der(X)t(X;4)	-	+	-
der(X)t(5;X)	-	-	+
Total Number of Translocations	14	12	11

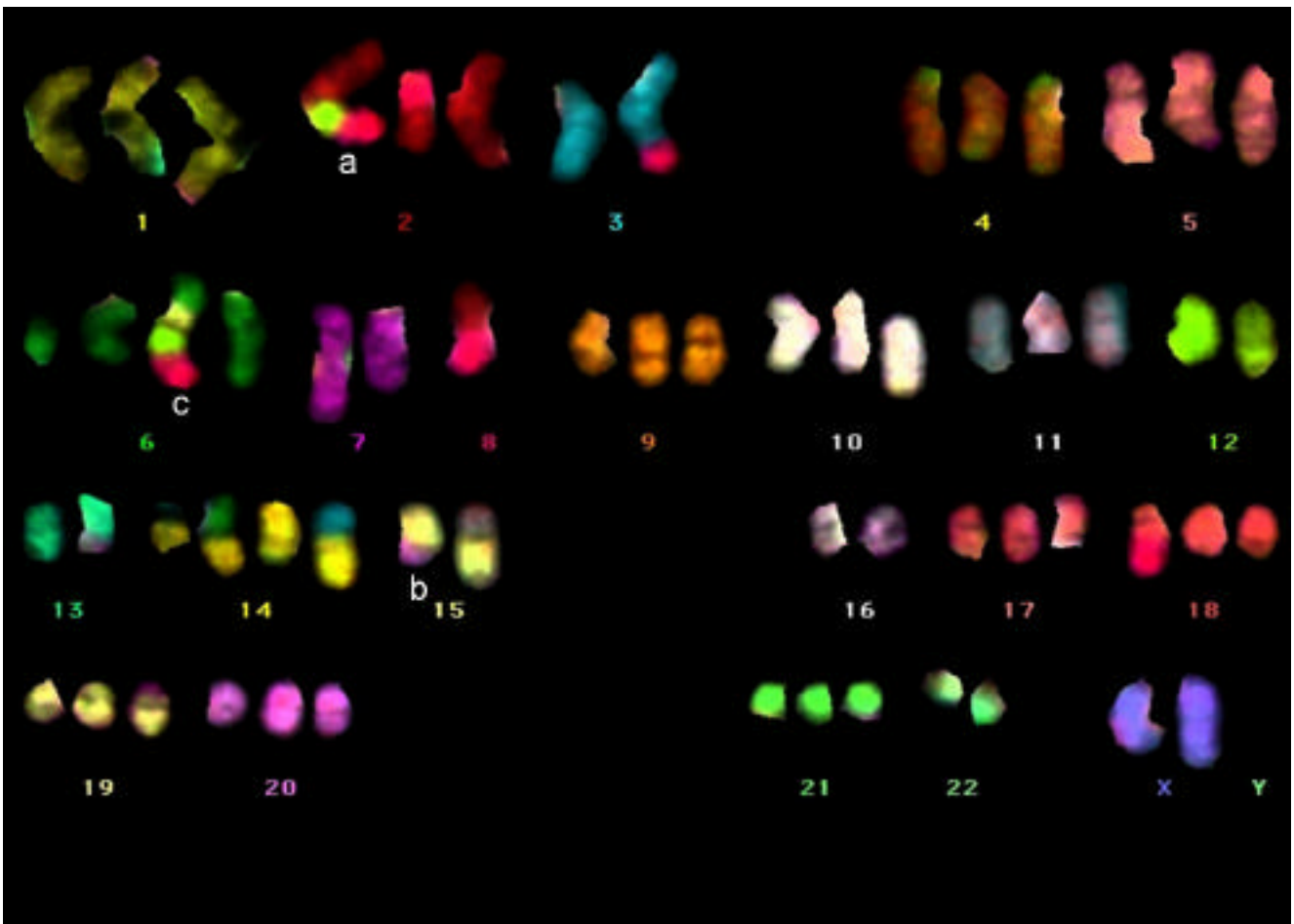


Figure 1. MFISH analysis of MDA-MB231(I) strain showing an example metaphase spread analysed using MFISH. The derivative chromosomes der(2)t(2;12;8) (labelled **a**) and der(15)t(20;15) (labelled **b**) were common to all three strains of MDA-MB231. The derivative chromosome der(6)t(6;19;12;8) (labelled **c**) was found only in strain I.

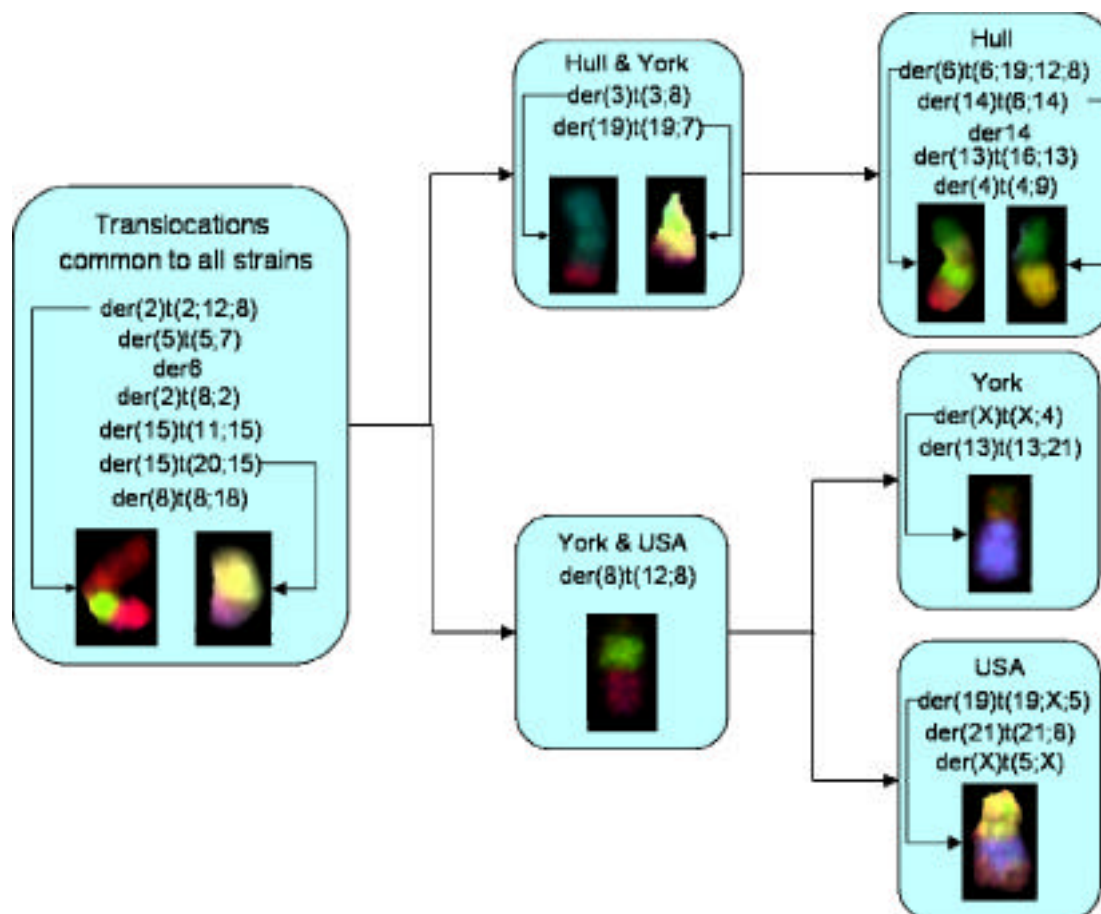


Figure 2. Flow chart demonstrating the possible karyotypic evolution of the MDA-MB231 cell line between individual laboratories.

IV. Discussion

The karyotypic evolution of established cell lines in culture has been demonstrated previously using MFISH (Bahia et al, 2002), comparative genomic hybridisation (CGH) (Jones et al, 2000) and conventional G-banding techniques (Mamaeva, 1998). However, this phenomenon had until now only been demonstrated in only one breast cancer cell line, namely MCF-7 (Jones et al, 2000; Bahia et al, 2002). It now appears that this type of evolution is not restricted to MCF-7, but also occurs in the MDA-MB-231 breast cancer cell line. The identification of karyotypic evolution in established breast cancer cell lines has important implications for their use as models in the study of cancer and cancer therapy. Thus, this phenomenon makes direct comparisons of data (especially cytogenetic data) between laboratories difficult and poses the risk of misinterpretation of results.

Despite this however, established breast cancer cell lines still have the potential to be good models of genetic change (Nugoli et al, 2003) provided the starting material is thoroughly characterised and adequate controls are in place. Such control measures include the parallel culture and analysis of control cultures to prevent misinterpretation of any mutations arising spontaneously. A complete cytogenetic analysis of the parent cell line (the direct ancestor of the experimental and control lines) should also allow direct comparisons of any karyotypic changes occurring after experiments. Cross contamination of cells lines can be monitored by the routine use of

methods such as short tandem repeat (STR) profiling. This system uses a series of primers to amplify the corresponding polymorphic loci, producing a numerical code representing the lengths of the PCR products produced. This system has previously been used to successfully identify a random panel of cell lines from cell banks around the world (Masters et al, 2001).

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References

- Ashman JNE, Brigham J, Cowen ME, Bahia H, Greenman J, Lind M, and Cawkwell L (2002) Chromosomal alterations in small cell lung cancer revealed by multicolour fluorescence in situ hybridisation. *Int J Cancer* 102, 230-236.
- Bahia H, Ashman JNE, Cawkwell L, Lind M, Monson JRT, Drew PJ and Greenman J (2002) Karyotypic variation between independently cultured strains of the cell line MCF-7 identified by multicolour fluorescence in situ hybridisation. *Int J Oncol* 20, 489-494.
- Gorzynski MJ, Leal RM, Mooberry SL, Bushweller JH, and Brown M (2004) Synthesis and evaluation of substituted 4-aryloxy- and 4-arylsulfanyl-phenyl-2-aminothiazoles as inhibitors of human breast cancer cell proliferation. *Bioorg Med Chem* 12, 1029-1036.

- ISCN (1995) An international system for human cytogenetic nomenclature. Mitelman F (Ed). Basel, Karger.
- Jones C, Payne J, Wells, D, Delhanty JDA, Lakhani SR, and Kortenkamp A (2000) Comparative genomic hybridisation reveals extensive variation among different MCF-7 cell stocks. **Cancer Genet and Cytogenet** 117, 153-158.
- Lacroix M, and Leclercq G (2004) Relevance of breast cancer cell lines as models for breast tumours: an update. **Breast Cancer Res Treat** 83, 249-289.
- MacLeod RAF, Dirks WG, Matsou Y, Kaufmann M, Milch H, and Drexler HG (1999) Widespread intraspecies cross-contamination of human tumour cell lines arising at source. **Int J Cancer** 83, 555-563.
- Mamaeva SE (1998) Karyotypic evolution of cells in culture: a new concept. **Int Rev Cytol** 178, 1-40.
- Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, Toji LH, Ohno T, Tanabe H, Arlett CF, Kelland LR, Harrison M, Virmani A, Ward TH, Ayres KL, and Debenham PG (2001) Short tandem repeat profiling provides an international reference standard for human cell lines. **PNAS** 98(14), 8012-8017.
- Nugoli M, Chuchana P, Vendrell J, Orsetti B, Ursule L, Nguyen C, Birnbaum D, Douzery EJP, Cohen P, and Theilliet C (2003) Genetic variability in MCF-7 sublines; evidence of rapid genomic and RNA expression profile modifications. **BMC Cancer** 3(1), 13.

- Watson MB, Bahia H, Drew PJ, Lind MJ, and Cawkwell L (2004) Chromosomal alterations in breast cancer revealed by multicolour fluorescence *in situ* hybridisation. **Int J Oncol** 24 (In Press).



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