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Common origins of MDA-MB-435 cells from various sources with those shown to have melanoma properties

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Abstract

Recently, the tissue origin of MDA-MB-435 cell line has been the subject of considerable debate. In this study, we set out to determine whether MDA-MB-435-DTP cells shown to express melanoma-specific genes were identical to various other MDA-MB-435 cell stocks worldwide. CGH-microarray, genetic polymorphism genotyping, microsatellite fingerprint analysis and/or chromosomal number confirmed that the MDA-MB-435 cells maintained at the Lombardi Comprehensive Cancer Center (MDA-MB-435-LCC) are almost identical to the MDA-MB-435-DTP cells, and showed a very similar profile to those obtained from the same original source (MD Anderson Cancer Center) but maintained independently (MDA-MB-435-PMCC). Gene expression profile analysis confirmed common expression of genes among different MDA-MB-435-LCC cell stocks, and identified some unique gene products in MDA-MB-435-PMCC cells. RT-PCR analysis confirmed the expression of the melanoma marker tyrosinase across multiple MDA-MB-435 cell stocks. Collectively, our results show that the MDA-MB-435 cells used widely have identical origins to those that exhibit a melanoma-like gene expression signature, but exhibit a small degree of genotypic and phenotypic drift.

Abbreviations: CGH – comparative genomic hybridisation; EGF – epidermal growth factor

Introduction

The MDA-MB-435 cell line was established from pleural effusion from a 31-year-old breast cancer patient who died in 1976 within one year of diagnosis [1]. The pathology showed residual tumour throughout the breast, draining lymphatics and axillary lymph nodes. Tumorigenicity and metastatic potential of the cell line in athymic nude mice was originally shown in 1990 [2, 3], and they have since been employed extensively as one of the most metastatic human breast cancer cell lines available (reviewed in [4, 5]).

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Controversy over the origin and/or provenance of these cells was sparked by the observation that the MDA-MB-435 cells used by the National Cancer Institute's Developmental Therapeutics Program 60 (NCI60) cell line screen (designated MDA-MB-435-DTP) exhibited a melanoma-like gene expression profile when subjected to gene array analysis [6]. Melanoma-like characteristics have since also been reported by others in terms of protease activity profiling [7], the expression of genes involved in melanin biosynthesis [8, 9], lack of expression of genes characteristic of breast cancer [9], and patterns of secreted proteins [10]. However, since there has been no direct link established between the MDA-MB-435 cells used in these studies and those used commonly in other laboratories, the possibility existed that the cells used in the NCI60 cell screen [6] were cross-contaminated with

a melanoma cell line and/or that they were of a different origin from the MDA-MB-435 cells used by most breast cancer researchers, including ourselves.

To test this possibility, we compared MDA-MB-435-LCC cells routinely used in our laboratories with those used in the NCI60 cell screens (MDA-MB-435-DTP) using microarray-comparative genomic hybridisation (CGH), genetic polymorphism genotyping and short tandem repeat fingerprint analysis, and used RT-PCR to confirm the expression of the melanocyte-specific tyrosinase gene among cell lines. We further compared the MDA-MB-435-LCC cells with MDA-MB-435 cells obtained separately from the MD Anderson laboratory source (MDA-MB-435-PMCC) using CGH, microsatellite analysis, chromosome number and gene expression profiling.

Our results collectively showed that the MDA-MB-435 cell lines from various sources are of the same origin, with slight changes being introduced as they evolve over time, consistent with phenotypic drift. They are genetically linked to MDA-MB-435-DTP cells, and thus all exhibit characteristics of a melanoma-like signature. We conclude that, based on current information, MDA-MB-435 cells are not a definitive model for breast cancer, although the possibility remains that they represent breast cancer cells which have undergone a transdifferentiation to melanoma-like cells or acquired melanoma features through lineage infidelity. They remain an important model for the study of various aspects of human cancer cell metastasis.

Materials and methods

Cell lines

MDA-MB-435 cells originally obtained from Dr Janet Price, MD Anderson Cancer Center in the late 1980s, and maintained by the Lombardi Cancer Center Tissue Shared Resource have been designated MDA-MB-435-LCC. MDA-MB-435 cells obtained from Dr Janet Price in 1997 and maintained at the Peter MacCallum Cancer Center, Melbourne, Australia have been designated MDA-MB-435-PMCC. MDA-MB-435/LCC6 cells, adapted to growth as ascites [11], were obtained from the Lombardi Cancer Center Tissue Shared Resource. The MDA-MB-435S cells were obtained from the ATCC (Manassas, Virginia) and the MDA-MB-435 cells used in the NCI60 cell screening program (designated here as MDA-MB-435-DTP) were obtained from Dr Dominic A. Scudiero of the National Institute of Health FCRDC. MDA-MB-468, MDA-MB-453, T47D, and SK-Br-3 cell lines were originally obtained from the ATCC. MCF-7_{ADR}/NCI/ADR-RES cells [12], were originally obtained from Dr Ken Cowan, NCI. The MDA-MB-435-LCC15 cells were previously designated LCC15-MB, but were recently shown to be MDA-MB-435-LCC (Thompson et al. [30]. PMC42-LA cells [13] were obtained from Dr Leigh Ackland, Deakin University, Melbourne, Australia.

Comparative genomic hybridisation (CGH) analysis

CGH was carried out according to methods described in detail elsewhere [14] using the same method as Thompson et al. [30]. The cell line and normal DNA samples were labelled during a DOP-PCR reaction, with digoxigenin and biotin and detected indirectly with anti-digoxigenin–fluorescein and avidin-rhodamine [31].

Microsatellite analysis

Cell line genomic DNA was analysed for nine microsatellite markers D7S518 (7q31), D7S666 (7q22.1), D8S300 (8q23.1), D8S326 (8q23.2), CYP19 (15q21), AIB1 (20q12), D22S284 (22q13.1), D22S307 (22q13.1) and D22S275 (22q12.1). The sequences of the primers are available at http://www.gdb.org/. The forward primers were labelled with a fluorescent dye (FAM or HEX, GeneWorks Pty Ltd, Adelaide, Australia). After PCR amplification, the products were denatured in 90% formamide and the alleles separated by electrophoresis through 6% denaturing polyacrylamide gels. The alleles were detected using a scanning laser fluorescence imager (Bio-Rad Molecular Imager FX). Six microsatellite markers on chromosome 8p were also tested (D8s550, D8s532, D8s1820, D8s136, D8s1706, D8s255) as described in Venter et al. (in preparation).

Ploidy analysis

Metaphase chromosome spreads were prepared from the cell lines using standard methods and the slides were stained with DAPI in phenyldiamine antifade solution. Metaphase chromosomes were viewed under a Zeiss fluorescence microscope and captured as described for CGH. The number of chromosomes in each of at least 25 spreads were counted using the Quips CGH karyotype program. The modal number of chromosomes and range was determined for each cell line.

Fluorescent in situ *hybridisation (FISH)*

FISH on metaphase chromosome spreads of the cell lines was as described in Venter et al. (in preparation). Ten probes on chromosome 8 were analysed; six probes were on chromosome 8p, three on 8q and one was at the centromere.

Expression array profiling

Cell lines were cultured under standard conditions using RPMI supplemented with 10% foetal calf serum. RNA collections were made (RNeasy kit,

Qiagen, Valencia, California) at mid-log growth phase and at ~80% plate coverage. RNA quality was gauged using spectrophotometric readings and the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, California). Research Genetics GF225 filter arrays (Research Genetics, Huntsville, Alabama) which contain ~5000 spotted 'breast-enriched' cDNAs were used. A standard protocol recommended by the manufacturer was followed for isotope labelling and interrogation using 4 μg (total) RNA. Following image analysis and data extraction (Pathways 3.0, Research Genetics), we applied selective filters to reduce the initial ~5000 gene set to a 3800-gene subset for the present analysis. This included removal of genes whose expression value was lower than a set threshold level and/or with low variance across all cell line samples analysed. An unsupervised hierarchical clustering algorithm was applied separately to the cell lines and genes, using the Pearson correlation coefficient as the measure of similarity and average linkage clustering [15]. The results are presented as dendrogram trees in which very similar elements are connected by short branches, and longer branches join elements with diminishing degrees of similarity. The gene cluster tree (vertical matrix axis) and gene IDs have been removed from the figure to enhance clarity, however gene IDs from each of the three more discriminatory clusters are shown in Table 6.

CGH-microarrays: AmpliOnc™ I array analysis

High molecular weight genomic DNA from the MDA-MB-435-DTP cell line was kindly provided by Dominic A. Scudiero. DNA from MDA-MB-435-LCC cells was extracted using the Qiagen DNeasy Tissue kit (Qiagen Inc., Valencia, California). Genomic DNA was labelled with Alexa-488 (green) dUTP and hybridised to the AmpliOnc™ I CGH-microarray in the presence of Alexa-594 (red) dUTP-labelled normal male reference DNA, according to the manufacturer's protocol (Vysis Inc., Downers Grove, Illinois), and as described in detail elsewhere [16]. Analysis of green/red intensity ratios allowed a modal DNA copy number to be calculated for each test DNA.

Genotyping for polymorphic drug metabolizing enzymes

Cell lines were assayed for genetic polymorphisms in the cytochrome P450 (CYP) 2C8, 2C9, 2C19 and 2D6 enzymes, and the (C3435T) polymorphism in the multidrug-resistance (MDR)/P-glycoprotein transporter using conventional polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP) assays as described in detail elsewhere [17].

Genetic finger printing

High molecular weight genomic DNA from the MDA-MB-435-DTP and the MDA-MB-435-LCC cells was sent to Orchid Cellmark (Nashville, Tennessee) for short tandem repeat (STR) PCR analysis.

RT-PCR analysis of tyrosinase gene expression

Tyrosinase gene expression was assayed by RT-PCR assay using standard methods. Briefly, total cellular RNA was prepared from the cells using RNAzol (Iso-Tex Diagnostics, Friendswood, Texas) according to the manufacturer's instructions. RNA was quantitated by absorbance at 260 nm and integrity was verified by gel electrophoresis. Samples (1 μg) of total RNA were subjected to reverse transcription into cDNA followed by PCR amplification of tyrosinase sequences using commercial RT and PCR reagents according to the manufacturer's instructions (Pharmacia, New Jersey). Tyrosinase specific primers (5′-TGT CAA TGG ATG CAC TGC TTG -3′ and 5′–GGT TTC CAG GAT TAC GCC GTA–3′) were synthesised by Integrated DNA Technologies (Coralville, Iowa), and the reaction resulted in the amplification of a 365 bp fragment from cDNA, but not from genomic DNA being separated by more than 12.7 kb in the genomic sequence.

Results

We obtained DNA from the MDA-MB-435-DTP cells arrayed by Ross et al. (2000) [6] to compare it with DNA from the same cell line which was obtained from the same original source (MDA-MB-435-LCC) and used in our institutions. AmpliOnc™ I CGH-microarray analysis indicated that the gene copy levels are almost identical between the two cell lines (Table 1). It is notable that the cells did not exhibit any of the gene amplifications typically found in breast cancers, such as c-Myc, erbB_2, EGFR or AIB1. In fact, neither the MDA-MB-435-LCC or MDA-MB-435-DTP cells showed obvious amplification of any of the 59 oncogenes measured by this CGH-microarray. We genotyped these cells, along with the MDA-MB-435/LCC-6 cell line variant, for a number of genetic polymorphisms in drug metabolising enzymes (Table 2). All three cell lines were homozygous for the wild-type alleles of CYP2C8, CYP2C9, CYP2C19 and CYP2D6*6, and, interestingly, all were homozygous for the rare CYP2D6*4 allele (Figure 1) as well as for the genetic polymorphism in the MDR1 gene.

Table 1. Gene copy numbers determined by CGH-microarray.

Cyto location	Gene	MDA-MB-435-DTP	Std dev	MDA-MB-435-LCC	Std dev
1p36.2-p36.1	FGR	0.94	0.05	1.11	0.11
1p34.3	MYCL1	0.90	0.06	1.13	0.17
1p13.2	NRAS	0.92	0.11	1.04	0.14
1q25-q31	LAMC2	0.89	0.05	1.18	0.05

Table 1. Continued.

Cyto location	Gene	MDA-MB-435-DTP	Std dev	MDA-MB-435-LCC	Std dev
2p24.1	MYCN	0.93	0.09	1.12	0.20
2p13-p12	REL	1.01	0.01	1.01	0.10
3p25	RAF1	0.88	0.05	0.77	0.03
3q26.3	TERC	1.18	0.02	1.56	0.18
3q26.3	PIK3CA	1.31	0.17	1.55	0.13
4q12	PDGFRA	0.84	0.04	1.11	0.10
6q22	MYB	0.89	0.03	0.97	0.06
6q25.1	ESR	0.90	0.13	0.96	0.03
7p12.3-p12.1	EGFR	0.83	0.01	1.22	0.09
7q21.1	PGY1	1.05	0.07	1.08	0.10
7q31	MET	1.36	0.18	1.03	0.02
8p22	CTSB	1.02	0.02	N.D	N.D
8p11.2-p11.1	FGFR1	1.03	0.03	1.03	0.02
8q11	MOS	1.00	0.02	0.77	0.01
8q24.12-q24.13	MYC	1.09	0.11	1.08	0.04
9q34.1	ABL1	0.99	0.10	0.84	0.05
10q26	FGFR2	0.98	0.01	0.85	0.06
11p15.5	HRAS	0.79	0.04	0.89	0.06
11q13	CCND1	1.01	0.03	0.98	0.02
11q13	FGF4/FGF3	0.96	0.04	N.D	N.D
11q13	EMS1	1.00	0.02	1.11	0.02
11q13.5-q14	GARP	1.08	0.03	1.20	0.16
11q13.5-q14	PAK1	1.11	0.04	1.08	0.10
11q23	MLL	1.09	0.05	N.D	N.D
12q23	CCND2	1.10	0.07	0.95	0.04
12p12.1	KRAS2	0.89	0.05	0.97	0.05
12q-q13	WNT1	0.84	0.07	0.86	0.04
12q13.2-q13.3	GLI	0.94	0.04	0.94	0.09
12q13.3	SAS/CDK4	0.92	0.06	0.87	0.06
12q14.3-q15	MDM2	0.93	0.10	0.96	0.07
14q32.3	AKT1	0.89	0.05	1.04	0.03
15q25-q26	IGFR1	1.11	0.02	0.98	0.04
15q26.1	FES	1.07	0.04	0.95	0.02
16p13.1	MRP1	1.03	0.05	0.75	0.03
17q21-q22	TOP2A	0.89	0.03	0.90	0.01
17q21.2	ERBB2	0.95	0.08	0.88	0.05
17q23	RPS6KB1	0.95	0.05	N.D	N.D
17q23	D17S1670	0.91	0.04	N.D	N.D
18p11.3	YES1	0.97	0.06	0.79	0.04
18q21.3	BCL23′	0.92	0.02	0.97	0.06
18q21.3	BCL25′	0.92	0.08	0.93	0.07
19p13.2	INSR	0.99	0.00	1.12	0.01
19p13.2	JUNB	1.03	0.02	0.82	0.07
19q13.1	CCNE1	0.87	0.03	0.85	0.10
20q12	AIB1	1.44	0.03	1.29	0.01
20q13	STK15	1.28	0.07	1.39	0.18
20q13	CSE1L	1.30	0.04	N.D	N.D
20q13.1	MYBL2	1.18	0.03	0.86	0.02
20q13.1-q13.2	PTPN1	1.32	0.06	1.34	0.06
20q13.2	ZNF217	1.22	0.06	0.95	0.07
21q22.3	CBFA2	1.17	0.07	N.D	N.D
22q11.21	BCR	1.01	0.05	N.D	N.D
22q12.3-q13.1	PDGFB	1.24	0.05	1.66	0.32
Xq11-q12	AR 5′	1.12	0.10	1.40	0.07
Xq11-q12	AR 3′	1.15	0.13	N.D	N.D

The genotype data prompted us to undertake genetic fingerprint analysis of the MDA-MB-435-LCC and MDA-MB-435-DTP cell lines (Table 3). MDA-MB-435-LCC cells were consistent with MDA-MB-435-DTP at 13 of the 14 loci, however at D7S820 the MDA-MB-435-LCC cells were homozygous [8] whilst the MDA-MB-435-DTP cells were heterozygous [8, 10]. In addition, MDA-MB-435-DTP cells have four loci with unbalanced alleles (peak height ratio $\leq$ 60%) and the MDA-MB-435-LCC cells are unbalanced at two loci. In total, these results indicate that the two cell lines originated from the same parental cell line and that a small degree of clonal drift has occurred.

CGH analysis of such drift between the MDA-MB-435-LCC and MDA-MB-435-PMCC cell lines showed similarities with gain of 3p14-qter, 6p11-cen, and 20q, and losses at Xp21-cen and Xq22-q24 (data not shown). Gains at 8q23-qter and 11cen-q13 and loss at 18p were unique to the MDA-MB-435-LCC cell line. The MDA-MB-435-PMCC cell line also had a unique change with gain at 7q22-qter.

Higher resolution fingerprinting of these cells lines by microsatellite analysis at nine markers on five chromosome arms confirmed the high degree of similarity between MDA-MB-435-LCC cells and MDA-MB-435-PMCC cells. The only difference was the loss of one of the alleles for two markers at 8q23 (D8S300 and D8S326 in MDA-MB-435-LCC compared to MDA-MB-435-PMCC (Figure 2). Higher density microsatellite analysis of chromosome 8p confirmed these differences seen on chromosome 8 between MDA-MB-435-LCC and MDA-MB-435-PMCC. All markers on 8p showed loss of one allele in the MDA-MB-435-LCC cell line (Table 4). On high density fluorescent in situ hybridisation (FISH) analysis, the MDA-MB-435-PMCC cells appeared to have three complete copies of chromosome 8. There appeared to be 2 normal chromosome 8s. The other chromosome 8 material was distributed over two chromosomes due to a translocation with a breakpoint between 8q22.1 and 8q24.1 (Figure 3). For the MDA-MB-435-LCC cell line, one of these four chromosomes had been lost leaving the two normal chromosome 8s and a chromosome with two copies of the 8q24.1 probe. This loss of one of the chromosomes in the MDA-MB-435-LCC cell line resulted in the LOH detected by microsatellite analysis.

MDA-MB-435-PMCC cells showed a modal number of 57 chromosomes (16 of 26 spreads), with a range of 44–59. The loss of one copy of chromosome 8 from the MDA-MB-435-LCC cell line was consistent with our observation of a decrease in the modal number to 52 chromosomes. The MDA-MB-435-LCC cell line has 52 chromosomes (9 of 26 spreads with 52; 7 of 26 with 51), with a range of 46–52 (Thompson et al. [30]).

The FISH results shown for the MDA-MB-435-PMCC cell line resemble the SKY results obtained by Davidson et al. [18] in Europe. Both methods show four

Table 2. PCR-RFLP genotype analysis of P450 (CYP) and MDR-1.

	CYP2C8*2	CYP2C8*3	CYP2C9*2	CYP2C9*3	CYP2C19*2	CYP2C19*3	CYP2D6*4	CYP2D6*4	PGP/MDR1
MDA-MB-435-LCC	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	*4/*4	wt/wt	VR/VR
MDA-MB-435-DTP	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	*4/*4	wt/wt	VR/VR
MDA-MB-435/LCC-6	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	*4/*4	wt/wt	VR /VR

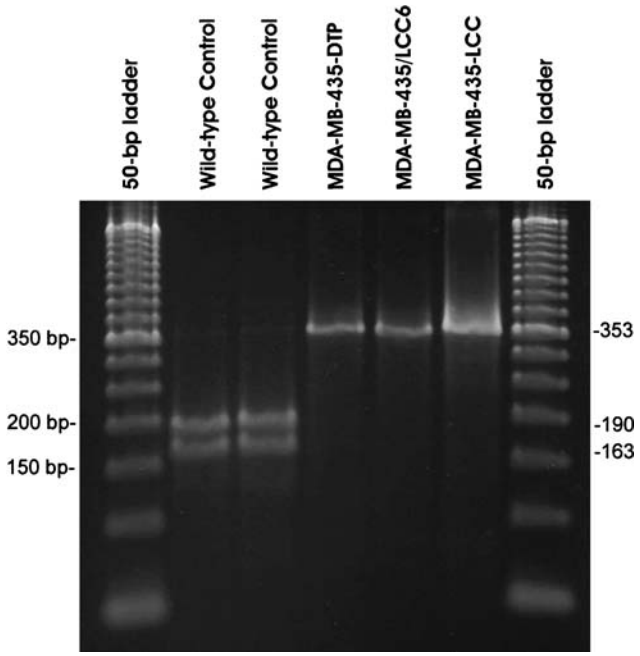

Figure 1. Cytochrome P450 2D6 genotyping of MDA-MB-435-LCC and MDA-MB-435-LCC-DTP. Genomic DNA was isolated from the cancer cell lines and used as template for PCR-RFLP genotyping. PCR products were digested and separated on a 2% agarose gel. The wild-type allele for CYP2D6 produces two bands, 190-bp and 163-bp in length while the CYP2D6*4 allele remains undigested (353-bp band).

Table 3. Results of short tandem repeat (STR) PCR analysis.

DNA STR Locus	MDA-MB-435-LCC	MDA-MB-435-DTP
D8S1179	13	13
D21S11	30	30
D18S51	13, 17	13, 17
D3S1358	14	14
VWA	16, (18)*	16, (18)*
FGA	21	21
D5S818	11, 12	(11)*, 12
D13S317	12	12
D7S820	8	8, (10)*
THO1	6, 7	6, 7
TPOX	8, (11)*	(8)*, 11
CSF1PO	11	11
D16S539	13	13
Amelogenin	X	X

( )*, peak height differences of ≤ 60%.

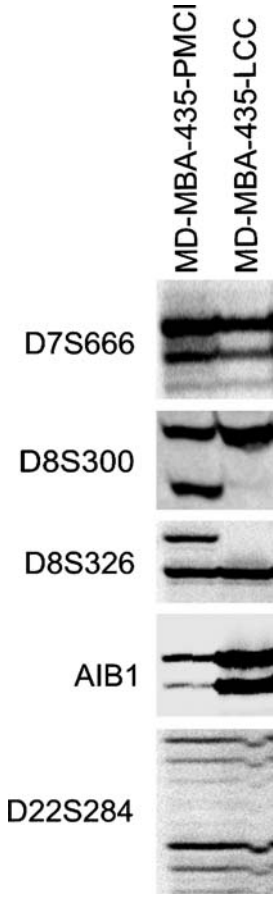

Figure 2. Microsatellite fingerprint comparison of MDA-MB-435-LCC and MDA-MB-435-PMCC. Microsatellite markers (indicted on the left of the figure) were PCR amplified and the alleles separated on a denaturing polyacrylamide gel. The fluorescently labelled DNA products were detected using a scanning fluorescence imager (Bio-Rad, FX Molecular Imager). Identical alleles were detected for all markers except that MDA-MB-435-LCC showed loss of heterozygosity for the chromosome 8q markers D8S300 and D8S326.

Table 4. Microsatellite analysis of chromosome 8p showing sizes of alleles in bp.

	MDA-MB-435-PMCC	MDA-MB-435-LCC
D8s550	256, 270	270
D8s532	244, 246	244
D8s1820	103, 109	109
D8s136	69, 73	69
D8s1706	272, 274	272
D8s255	116, 126	116

chromosomes containing regions of chromosome 8. The SKY results are consistent with the two chromosomes with only chromosome 8 material and also the chromosome 8 translocation detected by FISH. The chromosome count of both the Davidson et al. [18] study and the MDA-MB-435-PMCC give a modal number of 57. This is also the average modal number for all studies of

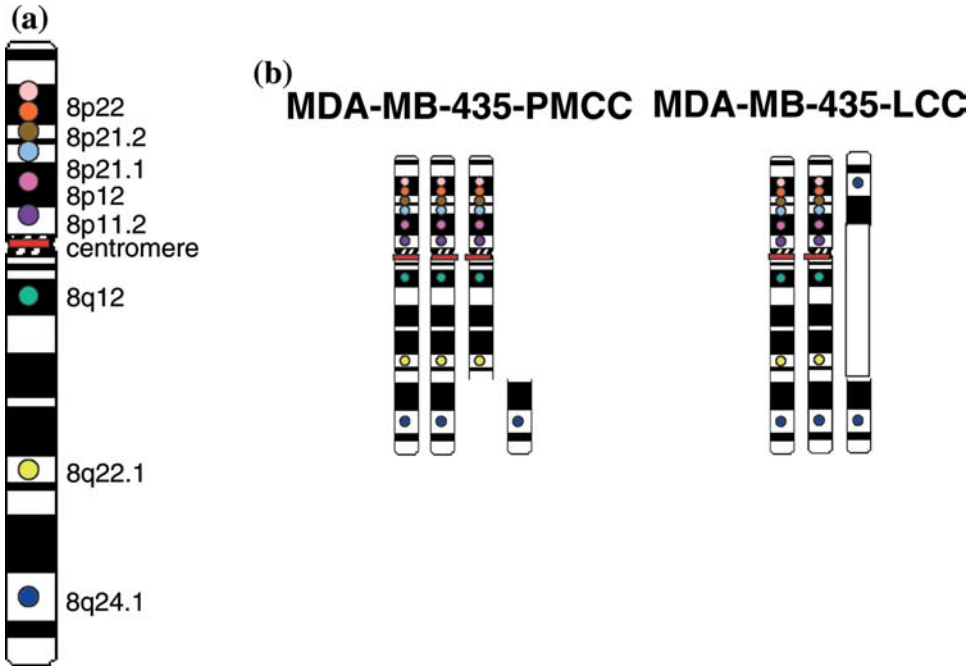

Figure 3. FISH analysis of MDA-MB-435-LCC and MDA-MB-435-LCC-PMCC. Each cell line was examined by FISH for chromosome 8. (a) Positions of the 10 chromosome 8 probes analysed by FISH. (b) Distribution of the chromosome 8 material in the two MDA MB 435 cell lines.

the MDA-MB-435 cell line (Table 5). The CGH profile obtained in the Davidson et al. [18] study most closely resembles the MDA-MB-435-PMCC profile as it shows gain of 7q and no gain of 8q or loss of 18p. These results suggest that the MDA-MB-435-PMCC cell line more closely resembles the MDA-MB-435 cell line studied worldwide, and that the MDA-MB-435-LCC is a variant cell line that has evolved over time to have minor changes. The karyotype of various versions of the MDA-MB-435 cell line are given in Table 5.

Gene expression profiling further supported the close relationship of MDA-MB-435-LCC and MDA-MB-435-PMCC cells (Figure 4). Each of these cell lines, and replicates thereof, were clustered together but distinctly from other human breast cancer cell lines arrayed (MDA-MB-468, MDA-MB-453, PMC42-LA ± EGF, NCI/ADR-RES, T47D, and SK-Br-3 cells ± EGF) as part of a larger study. As expected, duplicates of the MDA-MB-435 cell lines clustered together, as did the EGF-treated and -untreated PMC42-LA and SK-Br-3 cell lines. While this is an unsupervised visualisation of the data, the outcome is consistent with that reported by Ross et al. [6] in that MDA-MB-435 and its variants do not cluster with other breast cancer cell lines. Three specific subclusters were identified to illustrate the differential gene expression between the MDA-MB-435-LCC and MDA-MB-435-PMCC: the 'top cluster' is more abundant in MDA-MB-435-PMCC, while the 'middle' and 'bottom' clusters are more abundant in MDA-MB-435-LCC, consistent with our recent determination of the identity of MDA-MB-435-LCC15 cells as MDA-MB-435-LCC; these cell lines show very high levels of similarity. The gene identity of the elements in each of these clusters is shown in Table 6.

Having shown that the MDA-MB-435-LCC and MDA-MB-435-DTP originated from the same cell line but were not identical, we wished to confirm the expression of the melanocyte-specific tyrosinase gene. Tyrosinase expression was seen for all of the MDA-MB-435 sublines tested including the MDA-MB-435-LCC, MDA-MB-435/LCC6, MDA-MB-435-DTP, and the MDA-MB-435S subline obtained from the ATCC (data not shown), while MCF-7 and MDA-MB-231 cell lines were negative.

Discussion

We have shown that the MDA-MB-435-DTP cells used in the NCI60 cell screen were representative of those commonly used in the breast cancer research community. Our studies showed minor differences in both genetic variation and gene expression criteria between MDA-MB-435 cells obtained at different time points from the MD Anderson Cancer Center and maintained in an Australian research centre (MDA-MB-435-PMCC), maintained for a number of years at the Lombardi Cancer Center in Washington, DC, USA, and also transferred to the Thompson laboratory in Australia in 1997 (MDA-MB-435-LCC), or obtained from the NCI study (MDA-MB-435-DTP). We have also shown that the MDA-MB-435-PMCC cell line is very similar to the version of the cell line reported by Davidson et al. [18]. The CGH analysis in both these studies and the comparison of the FISH and SKY results for chromosome 8 support a common origin. Examination of the published karyotypes of the MDA-MB-435 cell line over time (Table 5)

Table 5. Karyotype analysis of MDA-MB-435 cell lines.

Chr.	MDA-MB-435 Satya-Prakash et al. [28] by karyotyping 14 markers (5 unidentified)	MDA-MB-435 Price et al. [29] by karyotyping 13 markers (1 unidentified)	MDA-MB-435 Leonessa et al. [11] by karyotyping 18 markers (3 unidentified)	MDA-MB-435 Davidson et al [18] by SKY 21 markers (All identified)	MDA-MB-435S (www.atcc.org), by karyotyping 19 markers (all unidentified)
1	1 × 3	1 × 2 t (1q;7q),del(1q−qter)	1 × 1 t(1q;7q), t(1p;?)	1 × 1, der(1)t(1;7), der(1)t(1;10)	1 × 2
2	2 × 3	2 × 3	2 × 2	2 × 2, del(2)(p?)	2 × 3
3	3 × 3 (3p−)	3 × 2 del(3p > pter), t(3p:?)	3 × 2 del(3)(q21:)*, del(3)(p21p24)*	3 × 2, del(3)(p?), der(3)t(3;19)	3 × 2, marker
4	4 × 2	4 × 2	4 × 2	4 × 2	4 × 2
5	5 × 2 (5p;13q)	5 × 3	5 × 3	5 × 2, del(5)(q?)	5 × 2
6	6 × 3 (6q−)	6 × 1 t(6q;?), del (6q > qter)	6 × 2 del (6)(q12q21)*	6 × 2, der(6)t(6;7)	6 × 0, three markers
7	7 × 3 (7p−)	7 × 2	7 × 2	7 × 2	7 × 1, six markers
8	8 × 2 (8q+)	8 × 2 t(8q;?)	8 × 1 (8q +)*, del(8)(p11p21)	8 × 1, del(8)(p), der(8)t(8;15)	8 × 2
9	9 × 3	9 × 3	9 × 2 t(9q;?)	9 × 2	9 × 2
10	10 × 2	10 × 2	10 × 2	10 × 2, del(10), der(10)t(10;18)	10 × 2
11	11 × 1 t(14q:11q) × 2	11 × 1 t(11p:?)	11 × 1 t(11q;14q)*	11 × 2, der(11)t(8;11)	11 × 0
12	12 × 2	12 × 2	12 × 2	12 × 2	12 × 2
13	13 × 1 t(?:13)	13 × 1 t(11q:13q)	13 × 1 t(11q;13q)	13 × 1, der(13) t(11;13)	13 × 1
14	14 × 2	14 × 2 (1 may be a marker)	14 × 1 (14p + (HSR))	14 × 1, der(14)t(1;14), der(?)t(14;16)	14 × 2
15	15 × 2	15 × 2	15 × 2	15 × 2	15 × 2, marker
16	16 × 2	16 × 2 t(11p:16q)	16 × 2	16 × 2	16 × 2
17	17 × 3	17 × 4 (1 may be a marker)	17 × 2	17 × 2	17 × 2, marker
18	18 × 2	18 × 2 (1 may be a marker)	18 × 2 del(18)(q12q22)	18 × 1, der(?)t(18;19)	18 × 1
19	19 × 2	19 × 2 (1 may be a marker)	19 × 1	19 × 1, der(?)t(6;19)	19 × 1
20	20 × 0 (20p+) × 2	20 × 0 t(20q;?)	20 × 0 (20q +)*	20 × 1, del(20), der(?)t(20;21) × 2	20 × 2, marker
21	21 × 1	21 × 2	21 × 1	21 × 1	21 × 1
22	22 × 1	22 × 2 t(22p;?) × 2	22 × 1 (22p +)*, (22p+) t(11p;22q)	22 × 2, der(22?)t(6;22)	22 × 0
X	X × 2	X × 2	X × 2 or 1	X × 2	X × 2
modal Chr # (range)	64 (59–67)	55–56	not done?	57 (44–62)	56 (55–62)

These marker chromosomes in MDA-MB-435 cell line described by Leonessa et al. [11] are identical to markers seen in Satya-Prakash et al. [28].

shows common marker chromosomes, suggesting that the current cell lines have evolved from those originally reported. These data address an important concern as to whether the MDA-MB-435-DTP cells, which were first shown to exhibit melanoma-specific gene expression, were truly representative of those used in other laboratories. Our data show commonality with representative stocks in North America, Australia and Europe. Also, the widespread and growing confirmation of melanoma-specific traits [7–10, 19] supports our observation that the MDA-MB-435-DTP cells are truly representative.

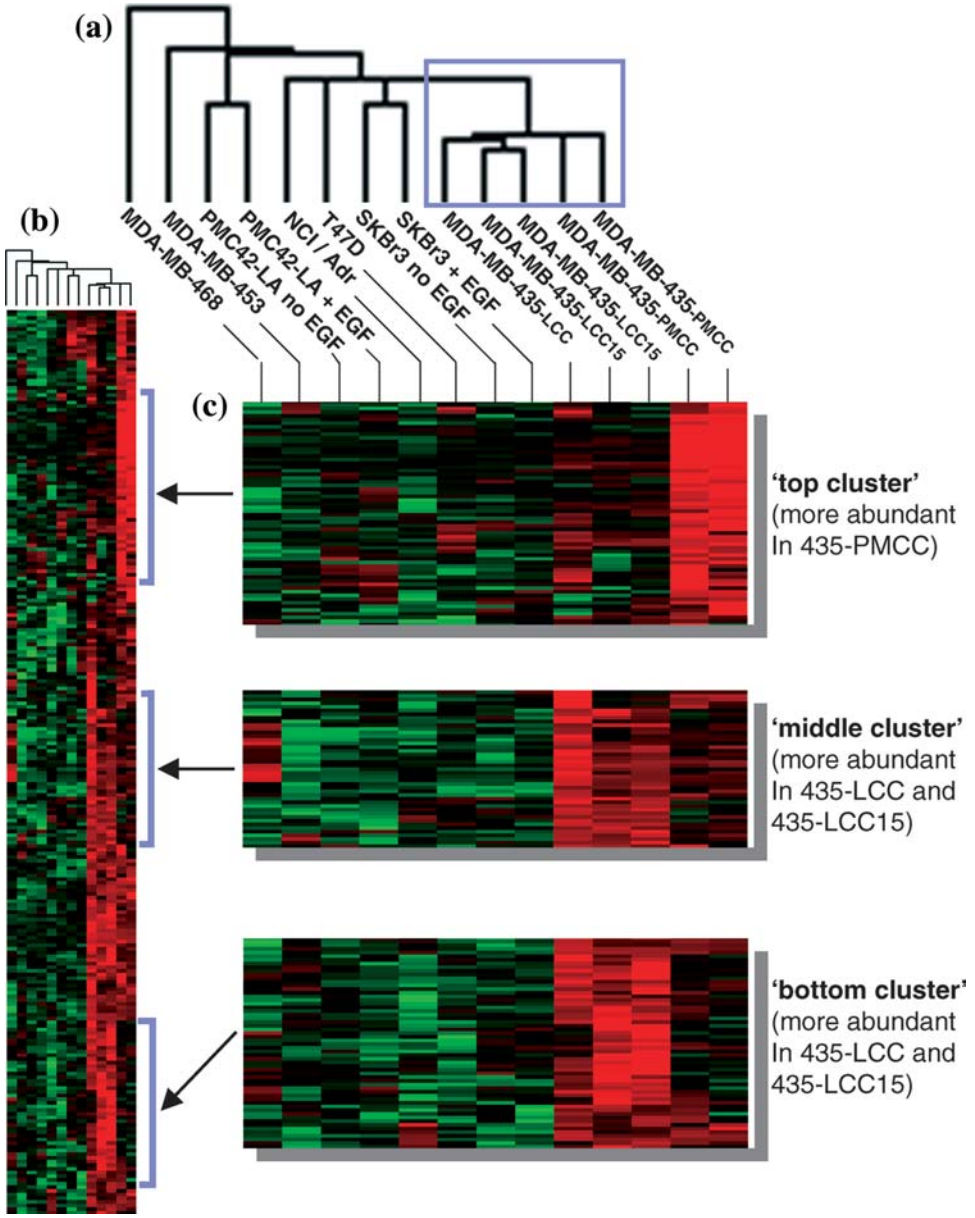

Figure 4. Microarray gene expression profiling of selected breast cancer cell lines. Two dimensional hierarchial clustering was applied to an expression dataset obtained from 5000 element (cDNA) Research Genetics 'breast arrays'. Shown at (a) is the derived cell-line dendrogram depicting similarity of lines based on a total of 3800 genes. Of note is that the MDA-MB-435 replicate collection and array interrogations for any individual line show greatest homology. At (b) is a subset of 453 genes which highlight the similarities and differences between MDA-MB-435 isolates. This is broken out into threee separate subsets at (c). The names of each element within the clusters at (c) are shown in Table 6.

Some variation in chromosome 8 was seen between the MDA-MB-435-PMCC and MDA-MB-435-LCC cells, with consistent changes seen by microsatellite analysis (low and high density) and high density FISH. Reproducible differences were also seen at the gene expression profile level, however, there were not localised to chromosome 8 and either represent additional genomic changes as detected by CGH, or indirect effects of these genomic changes on the transcriptome. The most likely explanation for this variation is that the MDA-MB-435-LCC cell line has undergone a passage-related loss of heterozygosity (LOH) for part or all of chromosome 8, and/or preferential expansion of this subpopulation from clonal heterogeneity at some point, although to our knowledge the cell lines used here were never purposefully cloned. The changes described above are reminiscent of the plethora of studies on phenotypic drift reported in a rat mammary adenocarcinoma system in the early 1980s [20–23], as also notably reported with different versions of the human mammary MCF7 [24] and T47D [4] cells lines where tremendous drift has been encountered, leading to many variants in different hands with different ER/PR status.

The MDA-MB-435 cell line has been the subject of considerable debate, since Ross et al. [6] showed that they clustered with human melanoma cell lines in a large scale expression profile analysis of 60 human cell

Table 6. Gene identifiers for cluster groups shown in Figure 4. Cluster ordered groups are presented here as depicted in Figure 4c. The IDs are based on Research Genetics Unigene Build 146 for 'breast specific' GF225 arrays.

Top cluster (more abundant in MDA-MB-435-PMCC)
MGC4276, HSPA9B, ATP1B2, TTF-I interacting peptide, THBS4, FXR2, ESTs, ACTA2, USP21, PMS2L5, C1D, DKFZp434P228, SCAMP2, CCR1, KIAA0215, CD68
ESTs, TNRC3, DKFZp434P0531, DKFZp761G2113, PCTK1, MINK, CLDN7, BPHL, KIAA1292, CALM3, MECP2, ESTs, FLJ20417, ESTs, CLASP1, LOC51064, PDGFRL, TNFRSF5, ESTs, ESTs, SHC1, APOD, COP9, FLJ20457, HTATSF1, KIAA0515, UXT
CYC1, ESTs, PEA15, DRPLA, CNOT7, EIF2S2, P311, ESTs, CELSR2, ESTs, PHLDA3, ESTs, FLJ23138, KIAA0016, CENPB
Middle cluster (more abundant in MDA-MB-435-LCC)
IFRD2, ESTs, IAA110, FLB5227 PRO1367, ESTs, KPNA1, DKFZP564O123, CYP27A1, CENPA, MYO10, MAGEA3, NOT56L, DSCR1, CFDP1, FLJ22704, KIAA0610, ACAA2, UP, ALDH1A1, MXI1, MAIL, CD44, SAT, SAT, SAT, PEPD,
HSPA4, FYN, SPRY2, procollagen-lysine, HMOX2, cDNA DKFZp434E082, KIAA0155, EIF1A, KIAA0678, SOD1, CGI-203, ubiquitin-protein ligase, MAPRE2, FLJ21778 fis, NLVCF
Lower cluster (more abundant in MDA-MB-435-LCC)
ERO1L, MGC4825, NBL1, SLC25A4, NFIC, MDS019, SAP30, C6orf5, RPL24, ESTs, SLC25A4, ESTs, FLJ14934 fis, AD036, GNG2, DREV1, RAP1, DDX24, MGC14156, KIAA0332, FLJ11342, NEDD5, SFRS4, MRPL34, HSP105B, ATOX1, ESTs, KIAA0326, GYG, LDHA, LDHA,SOD1, ESTs, UFD1L, PTP4A1, ITPKB, FLJ21007, MRPL3, CUGBP2, FLJ14753, RAB7

lines from 12 different organs of origin. Further evidence supporting the melanocytic origin of MDA-MB-435 cells is provided by Lee et al. [8] who showed that MDA-MB-435 xenografts expressed several genes involved in melanin biosynthesis and pigmentation including melan-A, ocular albinism 1 and tyrosinase-related protein 1. Jessani et al. [7] showed that the serine protease activity profile of MDA-MB-435 cells resembled melanoma cell lines rather than breast cancer cell lines, and MDA-MB-435 cells were shown by 2D gel electrophoresis profiling to resemble melanoma cell lines in their secretesome [10]. Ellison et al. [9] showed that MDA-MB-435 cells transfected with hepatocyte growth factor expressed several melanocyte-specific genes including ACP-5, tyrosinase-related protein and the melanocyte-specific gene tryrosinase. We confirmed here that MDA-MB-435-LCC, the ascites-derived MDA-MB-435/LCC6, MDA-MB-435-DTP and MDA-MB-435S cells expressed tyrosinase. It is interesting to note that Ellison et al. [9] did not find tyrosinase expression in the MDA-MB-435S cells which they obtained from the ECACC, and also found reduced levels of RXR and tyrosinase-related protein, again suggesting some drift among different holdings of the same cell line. In supplementary material (http://genome-www.stanford.edu/nci60/supplement.shtml) Ross et al. [6] indicated that MDA-MB-435S showed only part of the melanoma signature seen with MDA-MB-435-DTP, and using RFLP analysis confirmed that they were clonally related, to an extent seen in first degree relatives.

In the Ross study [6], MDA-MB-435-DTP and their $erbB_2$-transfected variant MDA-N clustered next to each other and within the group containing seven melanoma cell lines, co-expressing approximately 90 melanoma-specific gene products. Although this is a strong evidence supporting a melanocytic origin of MDA-MB-435 cells, it has been questioned due to the fact that not all of the breast cancer cell lines clustered into a distinct group. The three MCF-7 samples and the T-47D cells did cluster together, consistent with their oestrogen receptor-positive status and their requirement for oestrogen to grow, while the Hs578T and BT-549 cells clustered with CNS tumours and the MDA-MB-231 cells did not appear to cluster with any group. The Hs578T, BT-549 and MDA-MB-231 cell lines each show attributes consistent with epithelial to mesenchymal transition (EMT) [25], a phenotypic conversion deployed during development when epithelial cells need to move to another site, and a process that is gaining acceptance as a mechanism for carcinoma metastasis [26]. Another dramatic transdifferentiation similar to that seen with EMT is vasculogenic mimicry [27]. It is possible to speculate that the lack of clustering of the Hs578T, BT-549 and MDA-MB-231 cell lines represents such transitions, and a similar phenotypic transdifferentiation program may have occurred in the MDA-MB-435 cells, resulting in expression of a host of melanoma-specific genes. Recently, Sellappan et al. [19] showed expression of both melanoma-specific and epithelial-specific gene products in MDA-MB-435 cells, suggesting that lineage-infidelity may have occurred, and citing examples where this has been seen clinically.

Our own microarray analysis shows that the MDA-MB-435 cells cluster away from a variety of human breast cancer cell lines, and additional microarray studies of MDA-MB-435-LCC cells at the Lombardi Cancer Center show these cells to be less similar to breast cancer biopsies from patients than other widely used breast cancer cell lines (Clarke, manuscript in preparation). In total, these data imply that MDA-MB-435 cells are not representative of breast cancer, however, one cannot rule out the possibility that they represent the end result of a specific transdifferentiation such as seen with EMT or acquired through lineage infidelity. They retain certain mammary features, as detailed by Sellappan et al. [19], and although these

may continue to influence metastasis in a breast cancer-specific way, we feel that their limited resemblance to other breast cancers (including breast cancer cell lines and primary breast cancers) warrants caution in their use as a definitive breast cancer model.

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