Published in final edited form as:

Breast Cancer Res Treat. 2010 January ; 119(1): 249–251. doi:10.1007/s10549-009-0465-3.

Controversial *BRCA1* allelotypes in commonly used breast cancer cell lines

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To the editor:

The breast and ovarian cancer susceptibility gene *BRCA1* has been extensively investigated for its biological function and potential for targeted therapy. Cell lines derived from human tissues have been employed as research reagents in such studies, and one of those most commonly used is the breast cancer cell line MCF-7. Recently, the notion that MCF-7 carries a hemizygous wild-type allele of *BRCA1* has been referred to in a growing number of research articles [1–9], with some studies fundamentally based upon this concept. This idea seems to originate from an article published in 1996 by Holt *et al* [10]. In the article, Holt *et al.* noted that MCF-7 has only one allele at each of seven polymorphic DNA markers encompassing the *BRCA1* gene locus, indicating genomic loss of one copy of the 2 Mb region containing *BRCA1*. This description implies that MCF-7 carries only one copy of the *BRCA1* gene and seemingly has been interpreted as such in subsequent publications.

In agreement with Holt *et al.*, recent data from Genome-Wide Human Single Nucleotide Polymorphism (SNP) Array 6.0 analysis at the Wellcome Trust Sanger Institute (<http://www.sanger.ac.uk/genetics/CGP/>) revealed loss of heterozygosity (LOH) at the *BRCA1* gene locus at chromosome 17q21.31 in MCF-7. However, in contrast to the above-mentioned interpretation, these SNP array data suggest the presence of two copies of the *BRCA1* gene in MCF-7, with a normalized absolute copy number score 1.93 ± 0.412 (mean $\pm$ S.D.) at the markers within the *BRCA1* gene locus as calculated by Sanger Institute's PICNIC algorithm. In addition, we conducted a fluorescence *in situ* hybridization (FISH) analysis of the *BRCA1* gene locus using RP11-242D8, a BAC probe overlapping with the *BRCA1* 5' region. This assay was performed with two strains of MCF-7 cell line independently grown at distinct institutions (termed MCF-7-J maintained at Johns Hopkins University, and MCF-7-Aat Aichi Cancer Center Research Institute), and demonstrated that the modes of the *BRCA1* gene copy number in two strains were four and three, respectively (Fig. 1; b and c and Table 1). The same FISH result was obtained using another BAC probe, RP11-948G15, which was assigned at a

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Competing interests statement: B.H.P. is a consultant for GlaxoSmithKline and Horizon Discovery LTD. and has received research funding from GlaxoSmithKline..

close proximity to the 3' end of *BRCA1* (data not shown). The clonal variation between separate cultures of MCF-7 observed in our study is consistent with previous studies by others conducting spectral karyotype (SKY) analysis with MCF-7 [11–13]. Overall, MCF-7 cells were revealed to have multiple copies of *BRCA1* despite of the apparent LOH of the corresponding genomic region. This allelotype is generally considered to occur when one of the two alleles is initially deleted and the remaining allele is subsequently duplicated. Holt *et al.* additionally stated that another breast cancer cell line MDA-MB-157 was also subjected to genomic loss of one copy of the 2 Mb region containing *BRCA1* [10]. Similar to MCF-7, however, SNP array 6.0 data at the Sanger Institute indicates a copy number score 1.93 ± 0.412 (mean $\pm$ S.D.) at the *BRCA1* locus, suggesting that MDA-MB-157 has two copies of the *BRCA1* gene, although it also suggests LOH of the entire chromosome 17 in this cell line. Our FISH analysis suggested the presence of two major cell populations within our MDA-MB-157 culture, which carry two and four copies of *BRCA1*, respectively (Fig. 1d and Table 1). These results are again consistent with previous reports describing the karyotypic heterogeneity of MDA-MB-157 cell line [11, 12].

Collectively, these findings indicate that MCF-7 and MDA-MB-157 carry multiple copies of the *BRCA1* gene. Since the majority of cancer cells exhibit a chromosomal instability phenotype in which chromosomes are actively gained and lost, it should be noted that LOH does not necessarily mean the reduction of copy number at the corresponding genomic locus, particularly in cancer cells.

Acknowledgments

The authors thank Dr M. Seto for providing reagents and helpful discussion. This work was funded by grants from Department of Defense Breast Cancer Research Program Predoctoral Traineeship Awards BC083057 to M.M., the Ministry of Education, Science, Sports and Culture, Japan to Y.H., Flight Attendant Medical Research Institute (FAMRI) to H.K., and the Avon Foundation, Susan G. Komen for the Cure, the Mary Kay Ash Charitable Foundation, the Stewart Trust Fund, National Institutes of Health (CA109274 and CA88843), FAMRI, and the Breast Cancer Research Foundation to B.H.P.

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Figure 1.

Representative FISH photographs showing *BRCA1* gene copy numbers in two different cultures of MCF-7 (b, c) and MDA-MB-157 (d). Red signals generated by a BAC probe RP11-242D8 represent *BRCA1* gene loci, while green signals by RP11-443G13 represent Chromosome 17 pericentromeric region (17q11.2). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Procedures are described in Supplementary Materials and Methods. Chr. 17 pericen.: Chromosome 17 pericentromeric probe.

Table 1

FISH analysis of the *BRCA1* locus in MCF-7 and MDA-MB-157 cell lines.

	MCF-7-J			MCF-7-A			MDA-MB-157		
RP11-443G13 (17q11.2)	2	2	2	2	3	4	2	3	3
RP11-242D8 (<i>BRCA1</i>)	3	4	3	3	5	6	1	2	3
Cell number (%)	29	71	96	1	3	1	64	3	1