Inter- and Intraspecies Contamination of Human Breast Tumor Cell Lines HBC and BrCa5 and Other Cell Cultures

Abstract. *It is shown that the two most recently reported cell lines derived from malignant human breast tissue, HBC and BrCa5 are, respectively, rat and HeLa cell contaminants. The incidence of inter- and intraspecies contamination among 279 cell cultures from 45 laboratories in an 18-month survey is also presented.*

Among the many forces mobilized for the detection, cure, and prevention of breast cancer, a very important one has been the thrust to produce cell cultures from normal and malignant breast tissue for oncologic research in the laboratory. There are now available a number of cell lines derived from metastatic lesions or pleural effusions from cancer patients and a few from milk of normal individuals.

Numerous attempts to initiate cell lines derived directly from malignant human breast tissue have been recorded, but the success rate in establishing such cell lines is extremely low. To our knowledge, and according to the literature, there exist now only three such cell lines: BT-20 (*1*), BOT-2 (*2*), and Hs578T (*3*). In our studies of cultures of BT-20 cells (*4*) and Hs578T cells (*3*), the results conformed to those of their purported origin and the cultures had unique chromosomes. Cells of BOT-2 are not available as yet for confirmatory studies utilizing chromosome banding techniques (*5*).

During our effort to examine existing cell substrates to ascertain their adherence to purported biological origin (*4*, *6*–*8*), we reported earlier the HeLa cell contamination of four supposedly breast tumor–derived cell lines, HBT-3 and HBT-39b (*6*), ElCo and SH-2 (*8*). Cell lines SH-2 and SH-3 were originally initiated, respectively, from primary and metastatic tumor cells, drawn from two Caucasian women with breast carcinoma. The fact that both of these cultures subsequently exhibited type A isoenzyme mobility for glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49) (*9*), virtually unknown among Caucasians, had led us to study their chromosomes (*8*). Both cell lines revealed now well-defined HeLa marker chromosomes [for reviews see (*6*, *10*)]. Moreover, they possessed additional, identical marker chromosomes, observation of which was taken to mean that both cell lines were one and the same (*11*). One of the karyotypes, that for SH-3, studied by Miller and ourselves was subsequently published without reference to its having HeLa marker chromosomes (*12*). In it, HeLa marker No. 1 (in duplicate), No. 3 (in quadruplicate), and No. 4 are labeled M-4, M-19, M-18, respectively, while the additional markers possessed in common with SH-2 are labeled M-2, M-3, M-6, M-8, and M-12. Thus, SH-2 is another primary breast tumor culture which has been contaminated by HeLa cells.

We report here the discovery that the two most recently publicized cell lines, BrCa5 (*13*) and HBC (*14*) are of HeLa and rat origin, respectively. The BrCa5 cells were received from the originator at passage 335. They had been initially derived from a biopsy of an infiltrating duct carcinoma of a 93-year-old Caucasian woman (*15*). Chromosomes at the metaphase stage stained with trypsin-Giemsa to reveal the banding showed a range of 67 to 75 human chromosomes with a mode at 67 to 68. Among a series of rearranged or "marker" chromosomes we readily detected the HeLa marker Nos. 3 and 4. Number 3 was, as usual, present in several copies and No. 4 was clearly detectable, especially after fluorescent staining (*6*). Marker Nos. 1 and 2 both exhibited further rearrangements (Fig. 1). Mobility tests with G6PD revealed the fast-moving A type; that is, the type found in 25 percent of the Negro population, but rarely in Caucasians (*16*). These results are evidence that the BrCa5 cell line is in fact another strain of HeLa cells.

The designation HBC was originally given to a cell line initiated from an invasive duct cell carcinoma of a 77-year-old woman. A sample of HBC cells at passage 40 were studied upon receipt from its originators. Metaphases, on conventional staining, revealed at once an apparent nonhuman karyotype. The range of chromosomes was 54 to 62, with a mode at 57. While being aneuploid for the rat (*Rattus norvegicus*, $2n = 42$), a karyotype of these cells was compatible with that of the rat (*17*), but not of the human (Fig. 2). In addition, cell membrane immunofluorescence and zymogram mobility patterns for G6PD and lactate dehydrogenase were compatible

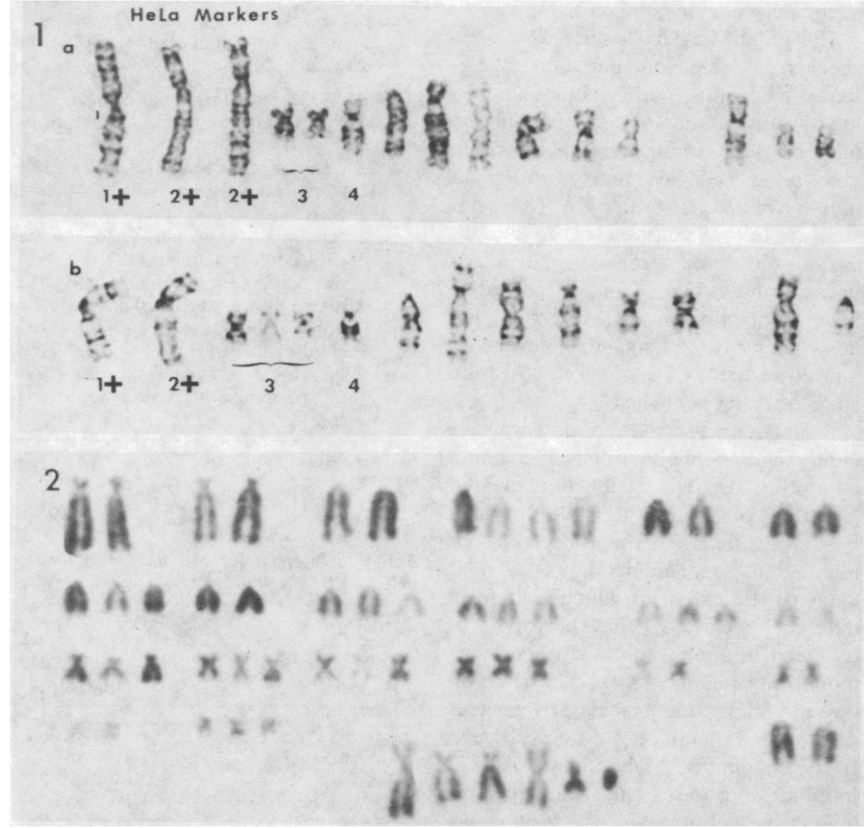

Fig. 1. (a and b) Marker chromosomes at the metaphase stage from two BrCa5 cells. HeLa marker Nos. 1 and 2 contain portions of chromosome Nos. 1 and 3 and 3 and 5, respectively, as previously reported (*6*, *10*), and additional rearrangements. Marker Nos. 3 and 4 are exactly as reported. Fig. 2. Metaphase chromosomes of an aneuploid HBC cell arranged according to the rat karyotype (*17*). Rearranged chromosomes are placed at the bottom center.

with those for rat but not human cells. The same results were obtained on two additional cultures of these cells received from a different laboratory; these cultures had been recovered from tumors induced in rats.

It was reported that the BrCa5 cells had "transformed spontaneously from fibroblastic to epithelial form in the 8th subculture" (*13*) and, in the case of HBC, "for about 3 weeks the cells were rounded and many were floating. During the 4th week, a burst of rapidly growing cells appeared, first in monolayers, but soon becoming multilayered with crisscrossing" (*14*). These quotations, from the original publications, describe occurrences during the course of cell cultivation which should serve as strong warnings of possible contamination for workers initiating and attempting to establish cell lines of human epithelial tissue origin and particularly of mammary duct lining cells.

It is currently a well-accepted concept that epithelial cells are difficult to grow (*18*), that human epithelial cells (normal and tumor) in general, and particularly excluding HeLa, grow relatively slowly (*19*), and that spontaneous transformation of human cells in vitro has not been adequately documented (*20*).

The extent to which intra- and interspecies cell line contamination is currently seen in laboratory situations during experimentation or cell line initiation may be seen from our experience in monitoring cell line purity during 18 months of testing (Table 1). These results are for cell cultures sent to our laboratory either for routine monitoring, or because the cultures were suspected of being contaminated. Of the errors, the great majority, fortunately, never advanced in the course of research to the stage of being published.

A good many cases of interspecies contamination involving cells in common laboratory use have been documented [see (*21*) for a review]. Table 2, which lists the kinds of interspecies contaminations encountered by us, indicates (i) the extent to which cells from different species are being cultivated; (ii) the need to apply rapid and continuous monitoring techniques (such as karyology and immunofluorescence for species determination) for cells from still relatively uncommon "laboratory species" in either short-term cultivation or after long-term culture when spontaneous chromosomal alterations may have occurred; and (iii) the possibility of inadvertently producing cell hybrids among these cells either in vivo or in vitro. Fortunately, new techniques which can better distinguish, for instance, between mouse and man chromosomes or fractions thereof in the same cell are being developed (*22*).

While recent publicity has again focused on the continuing contamination with HeLa cells, the potential for cross-cell contamination of both an intra- and interspecies nature is becoming an increasingly acute problem. We cannot overemphasize the need for workers with tissue cultures to adhere to the strictest culture procedures possible and to monitor cell lines regularly for purity by any of a number of publicized and relatively simple techniques. Finally, it is apparent that contributors to, as well as editors, referees, and readers of, scientific journals must attempt in concert to avoid disseminating data about or based upon the use of inaccurately specified cell cultures.

Note added in proof: We have shown that complexes of marker chromosomes can serve to characterize cell lines, particularly when they are heteroploid. The problem of distinguishing cell lines whether they are chromosomally altered or not but when they are from the same species is being solved also through continuing revelation of "genetic signatures" such as those afforded the cells by way of expression of enzyme polymorphisms (*23, 24*) and histocompatibility markers (*25*).

Table 1. Summary of karyology results on cultures submitted by 45 laboratories (6 February 1975 to 10 August 1976).

A total of 279 cultures was received	
Among 26 of these cultures	
2	were contaminated with microorganisms
23	did not grow at our laboratory
1	we are still trying to grow for banding
A total of 253 cultures was studied and	
41	of these cultures were not as purported
Among these 41 cultures	
21	were wrong species (see Table 2)
15	were HeLa instead of other human cells
1	was mixture of purported muntjak cells contaminating rat cells
1	was supposedly normal diploid human but was a BT-20 breast carcinoma cell culture*
3	cultures lacked one of two cells (one lacked rat in avian-rat mixture; two lacked mouse in human-mouse mixture)

*As determined by the chromosome banding technique.

Table 2. Interspecies cellular contamination detected by karyology.

Actual species	Purported species
Rat	Chicken
Hamster	Human
Hamster	Marmoset
Rat	Human
Mouse	Human
Rat	Monkey
Mongoose	Human
Dog	Horse
Dog	Mink
Hamster	Rat
Human	Gibbon
Mink	Human

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