

Correspondence

Re: M. Lippman, G. Bolan, and K. Huff. Three Papers on Hormones and Breast Cancer *in Vitro*. Cancer Res., 36: 4595-4601, 4602-4609, 4610-4618, 1976.

We have examined 4 cell cultures with the same designations as 4 of the 6 cell lines of human breast cancer studied by M. Lippman *et al.* These are HT-39, G-11, MDA-231, and MCF-7.

We obtained the first 3 cultures from Dr. D. Fine, who had just received them from Dr. G. Cannon; Dr. M. Rich sent us the MCF-7 cells. Of these cultures, the first 2 had the now well-publicized HeLa marker chromosomes, as well as type A or fast mobility for glucose 6-phosphate dehydrogenase, also characteristic of HeLa cells. Although differing slightly between cultures, the complex of marker chromosomes (G banding by trypsin-Giemsa) was practically identical with that which we reported earlier for a culture designated HBT39b, also a HeLa contaminant (8).

Cultures of MD-231 and MCF-7 each revealed their respective and unique banded marker chromosomes, which we had studied before (see Footnote c of Table 1 and Ref. 9).

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The purpose of this communication is 2-fold. First, we would like to inform the readers that "cell lines" HT-39 and G-11 are strains of HeLa cells. Second, it seems important to compare the results of tests obtained by Lippman *et al.* on these cells, derived as they are from an adenocarcinoma of the cervix, with 2 of the cultures derived from carcinomas of the breast to help delineate what might and might not be tissue-specific or normal *versus* neoplastic characteristics of cells in tissue culture. Table 1 summarizes data gleaned from the 3 publications regarding the 2 HeLa strains and 2 of the breast cancer-derived cultures discussed in those publications.

Most peculiar seems to be the discovery that all 4 cell cultures produced α -lactalbumin. Perhaps HeLa cells, regardless of their specific tissue source but because of long-term cultivation and highly rearranged chromosomes, have the potential to make this product, which *in vivo* is made only by mammary duct cells. In an apparently similar situation, "Chang liver cells," which are also HeLa derivatives

Table 1
Hormone responses of HeLa and breast carcinoma cells

Designation and origin of tissue	Chromosome markers, G6PD mobility	Paper 1				Paper 2				Paper 3	
		α -Lactalbumin	Estradiol (10^{-8} M) and tamoxifen (10^{-6} M) stimulation of synthesis	Estradiol (10^{-8} M) inhibition	Estrogen receptor activity	Progesterone receptors	Dexamethasone inhibition	Glucocorticoid-binding receptors	Epicortisol inhibition	Response to androgen	Detectable androgen receptors
Adenocarcinoma of cervix (= HeLa)											
Strain HT-39 (= HBT - 39b?)	HeLa, type A (Ref. 8 and this letter)	+	- ^a	+	Low	No	ND ^b	ND ^b	ND ^b	No	0
Strain G-11	HeLa, type A (this letter)	+	- ^a	+	Low	No	Highest	Intermediate	No	No	7.2
Breast carcinoma, pleural effusion											
Strain MDA-231	Unique, type B (previous communication ^c and this letter)	+	+ ^d	+	Low	No	Lowest	Lowest	No	No	0
Strain MCF-7 (cloned)	Unique, type B (Ref. 9 and this letter)	+	+	+	High	Yes	Intermediate	Highest	No	Yes	12.0

^a This lack of response had been noted earlier by these authors in these cultures, as well as in HeLa cells (5).

^b ND, not determined. HT-39 was apparently not used in Paper 2. There is instead a reference to a cell line 496 not otherwise identified.

^c Unique marker chromosomes observed in 1975 on culture from E. Jensen noted again in present culture.

^d Although not significant, an increase in thymidine incorporation was noted.

(7), give a weak positive reaction indicative of the production of tyrosine aminotransferase, characteristic of liver cells (1). Further, Chang liver cells, obtained directly from the originator and found to have characteristics of HeLa cells by this writer (7) and by Lavappa *et al.* (3), revealed in the work of Ludueña *et al.* (6) that 45% of the cells produce liver alkaline phosphatase. Although this is interpreted to mean that Chang liver cells are of liver origin, it is probable that they are no more of liver origin than the present G-11 and HT-39 strains of HeLa cells are of breast origin, although they are shown here to produce α -lactalbumin. The work of Colten (2) revealed unexpected results, namely, that some HeLa strains respond to a fetal serum factor by producing human, 4th component of complement (C4) and that the same was the case for all HeLa strains studied after exposure to a "C4-deficient factor" derived from guinea pigs. Lieblich *et al.* (4) showed that several strains of HeLa cells, when tested for the production of the " α " subunit of human glycoprotein tropic hormones, did so to a varying extent. This activity is said to be similar in nature to the "ectopic" production of this protein by tissue, which does not in normal conditions produce it. Finally, the work of Ofner *et al.* (10) with the "established human MA160 line" of "questioned prostatic epithelial cells," which was shown by us (7, 8) and by Zalta *et al.* (11) to be a strain of HeLa, revealed that the cells had the C₁₉-steroid-metabolizing enzymes and pathways of human prostate. Thus, it would appear that HeLa cells are aberrant human cells that can make a number of unexpected products, depending on the particular strain used and the stimuli applied.

Data on dexamethasone inhibition, glucocorticoid-binding receptors, and epicortisol inhibition of synthesis are not available for HT-39 (unless Culture 496 in the second paper by Lippman *et al.* represents this entity). For the hormone data shown, however, HT-39 is identical with MDA-231, and G-11 differs from it only in detectable androgen receptors.

We agree with the information provided by Dr. Nelson-Rees that cell lines identified as HT-39 and G-11 in fact represent HeLa contaminates. We appreciate his detailed characterization of these cell lines and totally concur with his findings. We no longer use these 2 cell lines as control cell lines in our studies. In subsequent studies other authentic human breast cancer cells have been used for comparative purposes (1, 2). Estrogen responsiveness, as measured by stimulation by physiologically relevant concentrations of estrogens and inhibition by antiestrogens, has only been observed by us with the authentic human breast cancer cell lines MCF-7 and ZR-75-1.

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However, both HeLa strains and MDA-231 are clearly different from MCF-7.

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Obviously, care must be taken so that reported results are for authentically defined human tumor cell lines.

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