Banded Marker Chromosomes as Indicators of Intraspecies Cellular Contamination

Abstract. *Chromosome banding revealed marker chromosomes characteristic of HeLa cells in cultures designated HEK, HEK/HRV, HBT-3, HBT-39B, MA160, and a strain of SA-4TxS-HuSa$_1$. Other HeLa cell characteristics found were glucose-6-phosphate dehydrogenase type A mobility and lack of the Y chromosome. Conventional chromosome analysis and immunological and enzymatic techniques serve to monitor species specificity and racial origin of the donor. Chromosome banding, however, can monitor intralinear karyotype peculiarity and its evolution during long-term cultivation.*

We have examined cell cultures presumably derived from a human embryonic kidney, two human breast tumors, a human prostatic adenoma, and a strain of human liposarcoma. We believe that they are, in fact, cervical carcinoma–derived HeLa cells, as judged by their karyologic and enzymatic characteristics.

Using conventional Giemsa staining, we first discovered a common marker chromosome in three presumably unrelated human cell lines, HEK (*1, 2*), HBT-3 (*3*), and HBT-39B (*4*). A similar marker had been described (*3*) among those characteristic of HBT-3 cells. We designated this chromosome the "Giemsa" marker (Fig. 1). It appeared slightly different in each cell line but always resembled the well-known marker chromosome of cercopithecid monkey cells (*5*).

Chromosome banding by the trypsin-Giemsa (*6*) and quinacrine mustard (*7*) methods revealed another common marker, not previously described. It consisted of a large isochromosome, twice the size of the Giemsa marker (Fig. 1). The banding pattern suggested that the Giemsa marker consisted of portions of a No. 21 chromosome and a No. 23 chromosome, while the large isochromosome contained duplicate portions of the long arm of chromosome No. 7.

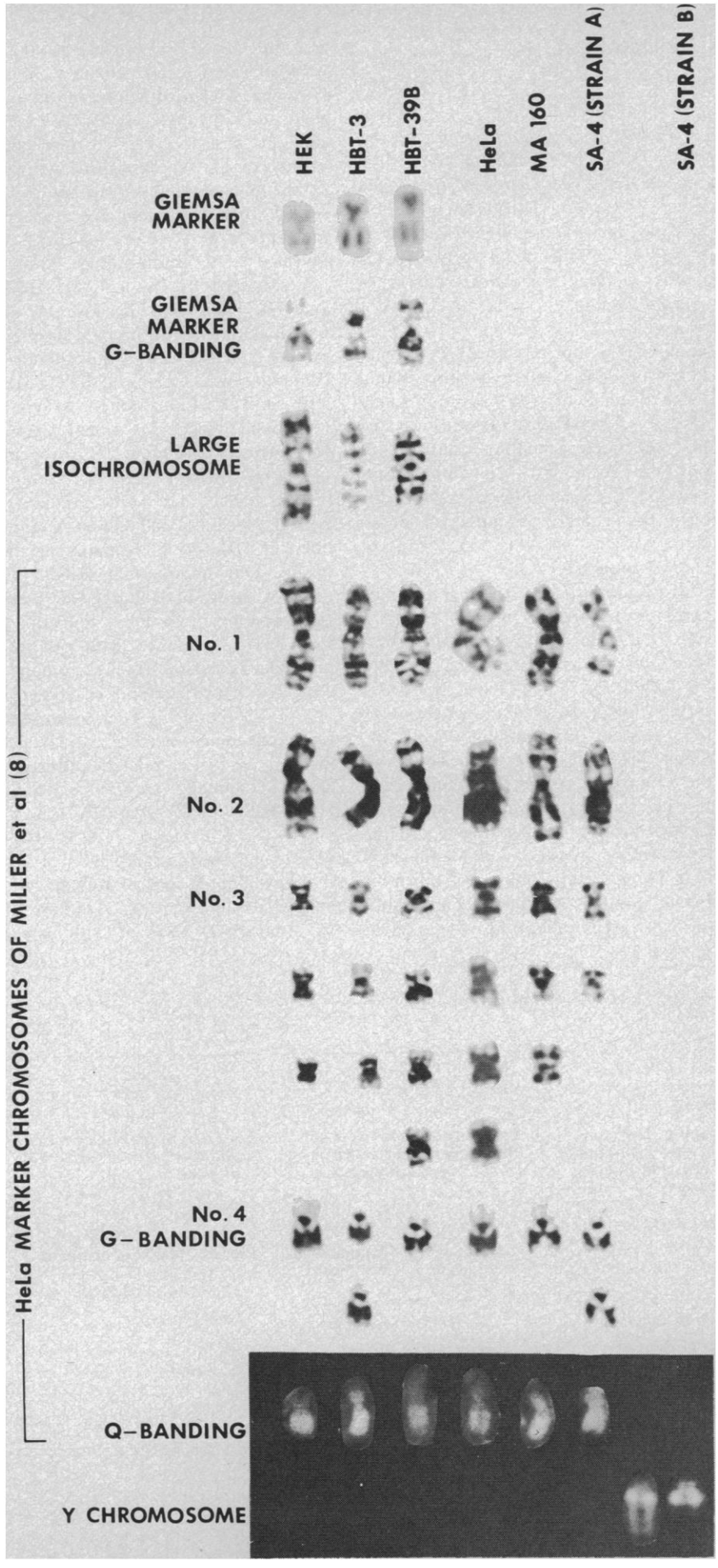
Most importantly, however, marker chromosomes that are peculiar to HeLa cells (*8–13*) also emerged. We concentrated on four such markers; possibly the same four that have been described under different designations in four separate publications (Table 1). Slight differences in the description of these markers reflect, no doubt, different interpretations and different HeLa cell strains studied. We chose the designations of Miller *et al.* (*8*) for these four known HeLa markers shown in Fig. 1. Neither the Giemsa marker nor the large isochromosome was noted in our HeLa cells. However, markers 1 to 4 appeared in all cell lines. Marker 3 is characteristically present in more than one copy per cell (*8, 9, 11–13*). HBT-3 cells also carried two copies of marker 4. As described by Miller *et al.* (*8*), marker 4 was readily observed after Q-banding by quinacrine mustard (Fig. 1). None of these cells revealed a Y chromosome. Although not expected in HeLa (*14*), HBT-3, and HBT-39B cells because of their female donor origin, it could have been present in HEK cells whose donor information was lacking. HBT-3, HBT-39B, and HEK exhibited a type A (fast band) isoenzyme mobility pattern for glucose-6-phosphate dehydrogenase (G6PD) which is also characteristic of HeLa cells and many cultures suspected of HeLa cell cross-contamination (*15–18*). Table 2 summarizes these results. Our finding that HBT-3 cells are most likely of HeLa cell derivation on karyologic grounds conforms to their nonconcordance with other cell lines derived from breast tumors in regard to ultrastructural criteria (*19*).

We also found the Giemsa and large isochromosome marker and HeLa markers in other, related cultures including human embryonic kidney HEK/HRV, with "adapted" Rauscher virus (*1*), a clone of HBT-3, HBTE (16c), and two passage levels of HEK from a different source from the original HEK studied (Table 2). Interestingly, although all cells of both passages

Table 1. Designations and descriptions of banded HeLa chromosome markers as designated and described by four investigators. The number 1 marker of Miller *et al.* corresponds to the number 2 marker of Francke *et al.*, the number 1 marker of Czaker, C of Walker, and so forth.

Miller *et al.* (*8*)	Francke *et al.* (*9*)	Czaker (*11*)	Walker (*12*)
Number 1 marker	*Number 2 marker*	*Number 1* marker*	*C marker*
Short arm and centromere of No. 1 chromosome and arm of No. 3 chromosome	Perhaps short arm of No. 3 chromosome with duplication or insertion and long arm of No. 3 chromosome	Long arm and centromere of No. 1 chromosome and long arm of No. 3 chromosome	Long arm of No. 1 chromosome and short arm of No. 3 chromosome
Number 2 marker	*Number 1 marker*	*Number 2 marker*	*G marker*
Probably short arm of No. 3 chromosome and long arm of No. 5 chromosome	Same as Miller's No. 2 marker	Long arm of No. 3 chromosome and long arm of No. 5 chromosome	Probably long arm and more of No. 3 chromosome and long arm of No. 5 chromosome
Number 3 marker	*Number 8 marker*	*Number 14 marker*	*H marker*
Small isochromosome in two or more copies	Isochromosome of short arm of No. 5 chromosome; multiple copies	Same as Miller's No. 3 marker	Same as Francke's No. 8 marker
Number 4 marker	*Number 6 marker*	*Number 13 marker*	*D marker*
"Dull" short arm† and long arm of No. 9 chromosome or No. 18 chromosome with bright fluorescence	Probably weakly staining short arm of No. 22 chromosome and long arm of No. 11 chromosome	Same as Miller's No. 4 marker	Long arm of No. 9 chromosome

* Czaker's description of these markers varies somewhat from Miller's, but it is stated that they "appear to be identical." † We believe that marker number 4 consists of weakly staining short arm of No. 22 chromosome and long arm and centromere of No. 12 chromosome.



of these HEK showed HeLa markers 1 to 4, only less than 2 percent of the metaphases of the cells that had undergone relatively few passages (low passage) revealed the Giemsa marker, whereas 80 percent of the cells that had undergone many passages (high passage) showed the Giemsa marker. The large isochromosome marker, on the other hand, was not observed in high passage of this culture of HEK while it was seen in low passage cells. The origin of this strain of HEK (low and high passage) has been described as follows, "sketchy, and the donor's sex and race are unknown" (*20*).

The Giemsa marker and large isochromosome marker are peculiar to HEK, HBT-3, and HBT-39B and related cultures, but have not been observed or reported in other HeLa strains and therefore can be considered markers for a group of closely related HeLa cell–derived strains not previously studied for banding characteristics. We presume (i) that the Giemsa marker originated in the HeLa-derived strain of HEK cells from the last source, increasing in frequency during passage; (ii) that high passage HEK cells with the Giemsa marker were the substrate for the "adaptation" of Rauscher leukemia virus to human cells (HEK/HRV) (*1*, *2*); and (iii) that, since HEK or HEK/HRV (or both) were disseminated to many laboratories, they are the most likely source of contamination of both HBT-3 and HBT-39B cultures and their derivatives now in existence.

We also examined the banding pattern of two unrelated cell lines, MA160 (*21*) and SA-4TxS-HuSa$_1$ (strain A and strain B) (*22*). Cell line MA160, derived from a Caucasian male, was previously suspected of being a HeLa contaminant because it had type A G6PD mobility (*15*, *16*) and lacked a Y chromosome (*14*). SA-4 (strain

Fig. 1. Selected marker chromosomes indicating closer affinity of HEK, HBT-3, and HBT-39B cells with each other than with MA160 and SA-4 (strain A), although all bear common HeLa markers. SA-4 (strain B) lacks all HeLa marker chromosomes, but appears to carry a translocation Y chromosome. Giemsa marker stained by conventional method (above) and trypsin-Giemsa (below). Marker No. 4 stained by trypsin-Giemsa (above) and quinacrine mustard method (below). The translocation Y chromosome (left) is compared with a normal human Y chromosome (right) from a cell of line MBA 9812, both stained with quinacrine mustard (× 1850).

A) derived from a male, race not specified (*22*), lacked a Y chromosome, had A-type mobility (*23*), and reacted just like HeLa in cytotoxicity tests for HL-A antigen (*24*). The banding pattern for these cells revealed markers 1 to 4 of HeLa, but had no Giemsa or large isochromosome marker (Table 2 and Fig. 1). SA-4 (strain A), like HBT-3, had two number 4 markers. In contrast, none of these markers was observed in SA-4 (strain B), which had type B (slow band) mobility for G6PD and Y chromosome fluorescence. This fluorescence was detected intercalated, however, into an acrocentric chromosome longer in size than a group D chromosome (Fig. 1) and was indicative perhaps of the permanence of such banding after translocation, as shown by Peterson *et al.* (*14*).

None of the markers discussed here was observed in seven additional tumor cell lines and one virus-transformed human embryonic cell line (*26–33*). These cells have been in culture for varying periods of time; they represent donors of both sexes, cells with high and low chromosome numbers, and cells with and without normal Y chromosomes and A- or B-type G6PD mobility. However, all of these cell lines including HT1080, a recent isolate with a pseudodiploid number of chromosomes, possess unique chromosome markers of their own in addition to a preponderance of normal human chromosomes.

Conventional chromosome staining and certain immunological and serological techniques are well suited to the characterization of species, sex, and racial specificity of diploid cell lines, or cells with minor karyologic alterations. However, chromosome banding techniques, and the marker chromosomes they reveal, offer a valuable tool for cell line monitoring and for the detection of cell line cross-contamination where donor's species, sex, and racial origin may be identical and otherwise indistinguishable or obscured because of changes inherent in long-term cultivation of cells.

We agree with Miller *et al.* (*8*) and Czaker (*11*) that similarities of marker chromosomes can serve to monitor the identity of cell lines. Although all related cell strains may not necessarily bear all the markers simultaneously, all strains have several markers in common.

It appears highly improbable that with time, in cultivation, all human

Table 2. Cell lines with new and previously described HeLa cell chromosome markers and cell lines without these markers. I, individual; C, company; n.d., not done.

Designation	Source	Tissue	Donor sex	Passage No.	Total chromosomes	Marker chromosomes*						G6PD
						Giemsa marker	Large isochromosome	HeLa marker of Miller *et al.* (*8*)			Y chromosome	
								No. 1	No. 2	No. 3	No. 4	
HEK	I (*1*)	Embryonic kidney	?	?	55–64	+	+	+	+	3 ‡	+	–	A
HBT-3	I (*3*)	Breast carcinoma	♀	30	43–70	+	+	+	+	3 ‡	2 ‡	–	A (*3*)
HBT-39B (clone 6)	I (*4*)	Breast carcinoma	♀	60	42–64	+	+	+	+	4 ‡	+	–	A
HEK/HRV	I (2)	HEK with virus	?	?	52–63	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	n.d.
HBTE (16c)	I (3)	Clone of HBT-3	♀	7–10	67–71	+	+	+	+	2 ‡	+	–	A (3)
HEK	C (*1*)	Embryonic kidney	?	31	57–61	+ (2%)	+	+	+	3 ‡	+	–	A
				76	49–66	+ (80%)	–	+	+	3 ‡	+	–	A
HeLa	ATCC (*25*)	Cervical carcinoma	♀	High	62–70	–	–	+	+	3–4 ‡	+	–	A
HeLa (E701-42B)	I (*25*)	Cervical carcinoma	♀	High	61–64	–	–	+	+	3 ‡	+	–	n.d.
MA160	C (*21*)	Transformed prostate adenoma	♂	106	61–70	–	–	+	+	3 ‡	+	–	A
SA-4TxS-HuSa$_1$ (strain A)	C (*22*)	Liposarcoma	♂	?	52–70	–	–	+	+	2 ‡	2 ‡	–	A (*23*)
SA-4 (strain B)	I (*22*)	Liposarcoma	♂	33	51–67	–	–	–	–	–	–	+ (translocation)	B
BT-20	I (*26*)	Breast carcinoma	♀	24	43–53	–	–	–	–	–	–	–	B
ALAB 496	I (*27*)	Metastasizing breast carcinoma	♀	18	53–57	–	–	–	–	–	–	–	B
734B	I (*28*)	Metastasizing breast carcinoma	♀	20	64–79	–	–	–	–	–	–	–	B
MDA MB-157	I (*29*)	Breast carcinoma	♀	11	60–65	–	–	–	–	–	–	–	B
MBA 9812	C (*30*)	Lung carcinoma ‡	♂	13	51–68	–	–	–	–	–	–	– and +	A (*14*)
Te 85	I (*31*)	Osteosarcoma	♀	20	46–57	–	–	–	–	–	–	–	B
HT 1080	I (*32*)	Fibrosarcoma	♂	9	44–48	–	–	–	–	–	–	+	B
HuE 13RS (a)	I (*33*)	Virus transformed	?	?	58–65	–	–	–	–	–	–	+	B

* See Fig. 1. † The number of copies. ‡ The diagnosis of the donor tissue in (*14*) should be carcinoma of lung instead of melanoma.

cells regradless of source would ultimately acquire identical and multiple chromosome markers, lose specific chromosomes, and exhibit a uniform isoenzyme mobility pattern.

Type A isoenzyme mobility for G6PD is a sex-linked characteristic associated almost exclusively with a fraction of the world's Negro population (*34*). Cell lines with this feature must be considered rare. Therefore, when this characteristic and absence of the Y chromosome (both features of HeLa cells) appears in any cell line, further analysis by chromosome banding techniques should be undertaken in order to rule out the possibility of HeLa cell cross-contamination.

Note added in proof: Most recently, cells of a culture of line RT-4, presumably derived from a bladder carcinoma of a human male (*35*), were examined and found to have approximately 90 chromosomes including the Giemsa marker, the large isochromosome marker, and the four markers of Miller *et al.* (*8*). They exhibited G6PD type A mobility and lacked a Y chromosome. They, therefore, resemble the HEK cells and are of HeLa origin.

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Acupunctural Analgesia? Evaluation by Signal Detection Theory

Abstract. *Pain responses to noxious thermal stimulation decreased in the acupunctured arm of subjects as compared to the arm not treated with acupuncture; this result suggested that effective analgesia had been induced. However, sensory decision theory analysis of the data revealed no difference in discriminability. This failure to find a sensory (physiological) change strongly suggests that analgesia had not been induced. The sole effect of acupuncture was to cause the subjects to raise their pain criterion in response to the expectation that acupuncture works.*

Is the reduction in the report of pain following acupunctural analgesia proof that amelioration of the pain experience has occurred, or is this decrease caused by suggestion that induces the subject to raise his criterion for reporting pain? This is not a new problem. A lengthy dispute surrounds the influence of hypnosis, placebos, anxiety, and "cognitive control" on perceived pain (*1*). Unequivocal proof of the effectiveness of these "psychic analgesics" has been difficult to obtain because the pain threshold is an unanalyzable amalgam of sensory (physiological) and attitudinal (psychological) variables. To answer the question one must resort to signal detection (or, more descriptively, sensory decision) theory (*2*). Sensory decision theory yields two measures of the subject's performance. The sensory or physiological parameter, d', provides the measure of sensory sensitivity or discriminability. The attitudinal or psychological index, L_x, indicates the subject's response criterion, that is, his willingness or reluctance to report the presence of pain. We report here a portion of a larger, continuing study on the effect of acupunctural analgesia on d' and L_x.

Two types of pain experiments analyzed by sensory decision theory, one on the effect of suggestion on L_x (*3*) and others on the effects of analgesics on d' (*4–6*), underlie the present study of acupunctural analgesia. The magnitude of the likelihood ratio criterion, L_x, reflects the subject's response bias or attitude toward uttering a pain response. Clark (*3*) found that administration of a placebo described to the subject as a potent analgesic sharply decreased the proportion of pain responses to noxious radiant heat stimulation, a result suggesting that the threshold for pain had been raised. However, analysis of the data according to sensory decision theory demonstrated that d' remained unaltered and that the sole effect of the placebo was to raise the subjects' pain criterion, L_x. Since d' did not decrease, he concluded that the placebo had not diminished the subjects' sensory experience; that is, analgesia had not been produced.

The other sensory decision theory measure, d', provides a relatively pure index of sensory sensitivity or discriminability that remains unaltered when nonsensory variables such as attitude, expectation, and motivation are changed. A low d' means that the subject tends to confuse stimuli of lower