Henrietta Lacks, HeLa Cells, and Cell Culture Contamination

Brendan P. Lucey, MD; Walter A. Nelson-Rees, PhD†; Grover M. Hutchins, MD

• Henrietta Lacks died in 1951 of an aggressive adenocarcinoma of the cervix. A tissue biopsy obtained for diagnostic evaluation yielded additional tissue for Dr George O. Gey's tissue culture laboratory at Johns Hopkins (Baltimore, Maryland). The cancer cells, now called HeLa cells, grew rapidly in cell culture and became the first human cell line. HeLa cells were used by researchers around the world. However, 20 years after Henrietta Lacks' death, mounting evidence suggested that HeLa cells contaminated and overgrew other cell lines. Cultures, supposedly of tissues such as breast cancer or mouse, proved to be HeLa cells. We describe the history behind the development of HeLa cells, including the first published description of Ms Lacks' autopsy, and the cell culture contamination that resulted. The debate over cell culture contamination began in the 1970s and was not harmonious. Ultimately, the problem was not resolved and it continues today. Finally, we discuss the philosophical implications of the immortal HeLa cell line.

(*Arch Pathol Lab Med.* 2009;133:1463–1467)

HENRIETTA LACKS

On February 1, 1951, a 30-year-old woman named Henrietta Lacks presented to the Johns Hopkins Gynecology Clinic in Baltimore, Maryland, for symptoms of spotting between her menstrual periods. Her last menstrual period had been on January 4, 1951.[1] Although the results of her general examination were unremarkable, examination of the cervix revealed a raised, smooth, glistening, and purple lesion less than 2.54 cm (1 inch) in size.[2] The lesion was confined to the cervix and appeared different from other carcinomas of the cervix seen by the treating physician. It was later noted in the autopsy report[3] by Ella Oppenheimer, MD, that ''1 year before death the patient delivered a normal infant and 6 weeks later her cervix was said to be normal. Three months later she presented herself to the clinic with a 2–3 cm cervical tumor.'' Results of tests for sexually transmitted diseases were negative and a biopsy of the cervix was performed. Four pieces of tissue from the biopsy were sent to the pathology department and ''epidermoid carcinoma, cervix uteri, spinal cell type'' was diagnosed with definite invasion of the stroma (Figure 1).[1]

During the next several months, the patient received 4800 mg-h of radium and 11 500 R (roentgen) of deep x-ray.[3] Treatment failed to prevent spread of the cancer, however, and it extended relatively rapidly to both parametria. On August 8, 1951, she developed severe abdominal pain and was admitted to The Johns Hopkins Hospital (Baltimore, Maryland). Her pain became progressively more severe and intractable. Because of failure to void urine, ureteral catheterization was unsuccessfully attempted several times and the serum level of nonprotein nitrogen rose to from 120 to 150 mg/dL (reference range, 25-50 mg/dL). Diathermy therapy was tried without positive effect. Henrietta Lacks died at 12:15 AM on October 4, 1951.[3]

THE AUTOPSY

Ms Lacks' autopsy was performed at 10:30 AM on the same day as her death. Examination of the body revealed a ''well-developed, thin, colored female [with] deeply pigmented skin over the lower abdomen such as seen after x-ray treatment.''[3] The peritoneal cavity contained a small amount of yellowish fluid and approximately 1 L of fluid was found in the pleural cavity, but the pericardium was devoid of fluid. The lungs were noted to have bibasilar lobar pneumonia with cheesy material in the bronchi. The mucosa of the bronchi was blood stained. The cranial cavity and neck organs were not examined because permission was not granted.

Small, white, and firm nodules were observed throughout both the thoracic and abdominal cavities, including the surfaces of the peritoneum, the entire length of the intestines, and the surface of the liver. Furthermore, both the pleural surface and the superior surface of the diaphragm (right side more than the left side) were covered with nodules, as were the lung, liver parenchyma, and the pericardium. The nodules varied slightly in size, measuring from 8 mm in diameter on the peritoneal surface to 1 cm in the lung parenchyma. However, the largest mesenteric lymph node infiltrated with tumor was 6 cm in length. Small tumor nodules, 3 mm in diameter, were seen in each adrenal gland. At the apex of the right ventricle, a tumor nodule approximately 1 cm in diameter protruded into

Accepted for publication March 6, 2009.

From the Department of Neurology, Michael O'Callaghan Federal Hospital, Nellis Air Force Base, Nevada (Dr Lucey); and the Department of Pathology, Johns Hopkins Hospital, Baltimore, Maryland (Dr Hutchins).

† Deceased. Dr Nelson-Rees, retired, contributed significantly to the manuscript before his death.

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Brendan P. Lucey, MD, Department of Neurology, Michael O'Callaghan Federal Hospital, Nellis Air Force Base, 1400 N Las Vegas Blvd, Nevada 89191 (e-mail: brendanlucey@hotmail.com).

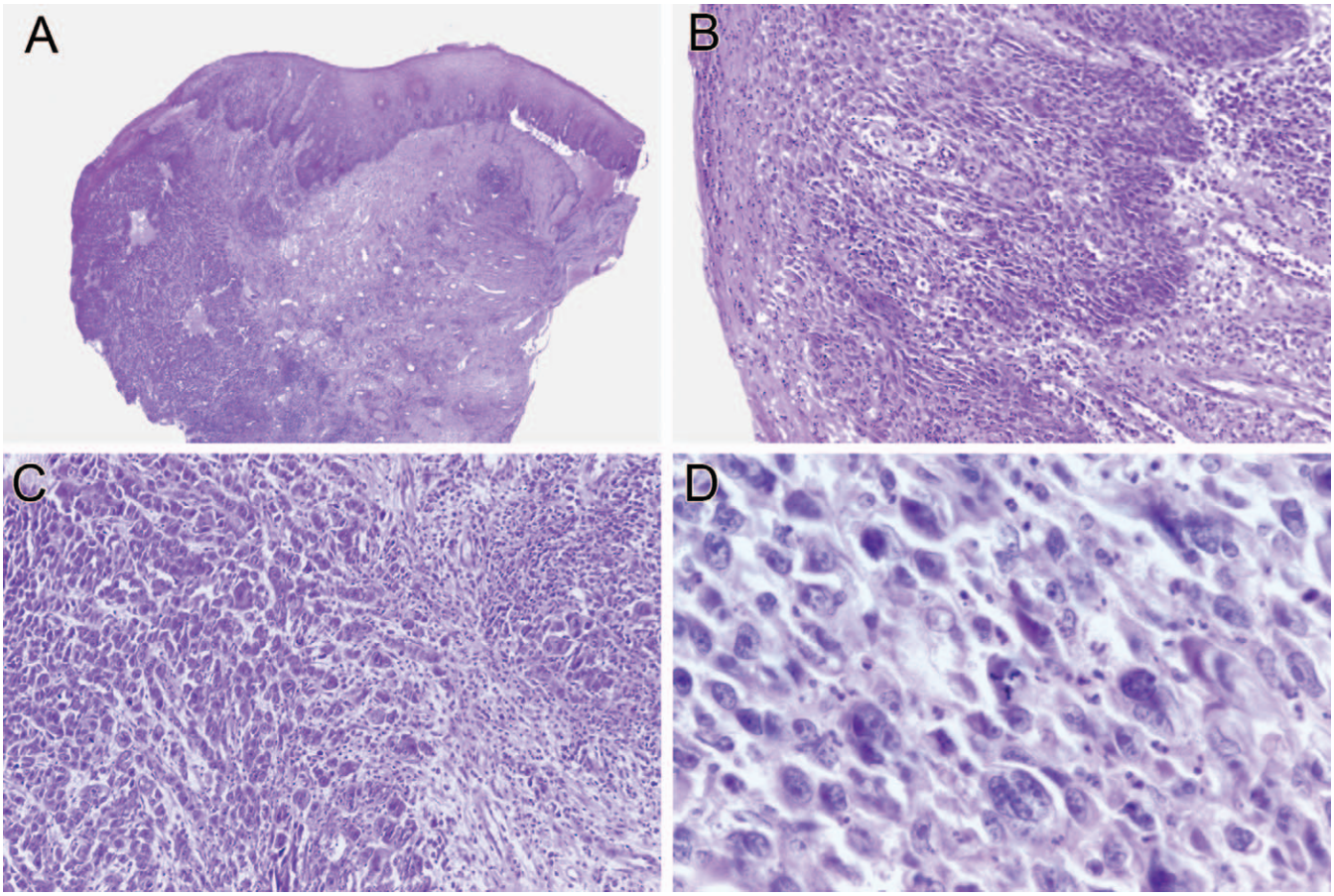

Figure 1. *A, Overview of a portion of the biopsy specimen taken from the cervix of Ms Henrietta Lacks. The squamous epithelium at upper right appears normal. The portion of epithelium at the upper left contains carcinoma in situ, shown in B. The part of the specimen at lower left shows infiltrating carcinoma, shown in C. B, Carcinoma in situ with an inflammatory cell reaction in the stroma. C, Infiltrating carcinoma. D, Infiltrating carcinoma showing marked pleomorphism of the malignant cells (hematoxylin-eosin, original magnifications ×5 [A], ×64 [B and C], ×100 [D]).*

the lumen. Relatively little necrosis was seen in any of the nodules.

A large subcapsular hematoma was present at the superior pole of the right kidney and a tumor nodule had grown into the capsule. Bilaterally, the ureters, calyces, and pelves were markedly dilated, consistent with severe hydronephrosis. The left ureter was involved in a mass of tumor just inside the brim of the pelvis, while a tumor mass near the posterior wall of the bladder entangled the right ureter. The bladder itself was adherent to the anterior abdominal wall. Many small nodules were seen on the bladder mucosa, and the external surface was nearly a solid mass of tumor.

The right ureter was dilated within 4 cm of the bladder, where the dilatation ceased abruptly. At this level, the circumference of the ureter was 14 mm; distally, the right ureter had been left intact and a probe passed with some difficulty down to the bladder. The probe could not be passed through the left ureter to the bladder, although both ureteral openings appeared patent from within the bladder. Closer examination revealed that the left ureter was dilated to the bladder wall, at which point a mass of tumor on the external surface caused the obstruction. The bladder was partially surrounded by nodular masses of tumor that penetrated the bladder wall, particularly in the trigone area. The bladder was not especially dilated. Tumor was seen infiltrating the wall of the vagina and friable masses of tumor replaced the cervix. The uterus was approximately normal in size and covered with tumor nodules, while the fallopian tubes and ovaries were obliterated by clusters of tumor nodules. A mass of tumor surrounded the iliac veins, and the area of the right iliac vein appeared to have tumor entering its lumen. Focal uremic diphtheritic colitis was also noted.

HeLa CELLS

Henrietta Lacks' cervical biopsy supplied tissue to the pathology department for clinical evaluation and to the Tissue Culture Laboratory in the Department of Surgery at The Johns Hopkins Hospital for research purposes. In 1951, George Gey, MD, was director of the laboratory and had already spent many years at Johns Hopkins as a student and faculty member. Prominent scientists at Johns Hopkins, such as Ross Harrison, MD, PhD, and Warren Lewis, MD, made important contributions to the history of tissue culture.[4,5] Dr Gey and his wife and chief collaborator, Margaret Gey, RN, continued in this tradition and began working on tissue culture in association with Dr Lewis in 1922. Dr Gey's work grew to encompass in vitro investigations related to endocrinology, cancer, and virology in addition to intracellular and membrane cytology.[4] However, his greatest scientific contribution was due to Henrietta Lacks.

While Henrietta Lacks was treated at Johns Hopkins, Dr

Figure 2. *Seated top left, clockwise are Mary Kubicek, George O. Gey, MD, and Walter A. Nelson-Rees, PhD, at a conference in 1970.*

Gey was attempting to fulfill ambitious goals for the Tissue Culture Laboratory, that is, ''the isolation and maintenance of normal and malignant or otherwise diseased tissues as temporary or stable organoids or as derived cell strains.'' [6] Toward this purpose, Dr Gey and his colleagues collected tissue from surgical procedures throughout the hospital.[7] Approximately 30 specimens of cervical cancer had been sent to the laboratory of Dr Gey by the time Ms Lacks presented to the gynecology clinic.[2] An investigator in the laboratory, Mary Kubicek (Figure 2), placed cells obtained from the biopsy specimen of Henrietta Lacks into culture by using the roller-tube technique; the cells grew robustly, contrary to the results with previous specimens, becoming the first human cancer cell line immortalized in tissue culture. The cells were named ''HeLa'' after the initial 2 letters of Henrietta Lacks' first and last names. She would not be credited as the originator of the cell line for many years, and HeLa cells were misinterpreted as originating with ''Harriet Lane'' or ''Helen Lane'' for years.[7,8]

Previous efforts to grow either normal cervical epithelium or cervical carcinoma in culture proved elusive[9]; however, efforts to grow cells from the aggressive adenocarcinoma of the cervix that had affected Henrietta Lacks were successful. Twenty years later, reexamination of the histopathology slides from Ms Lacks' surgical biopsy and autopsy led to a revision of the initial diagnosis, with the finding that the patient had a very aggressive adenocarcinoma of the cervix.[9] The cervical carcinoma was clearly very malignant and the patient had a rapid clinical deterioration. Although the concept of rapidly progressive cervical carcinoma has been questioned,[10] this case history would suggest otherwise. Recently, HeLa cells have been shown to contain human papillomavirus (HPV) 18 DNA[11] and HPV18-positive HeLa cells have been linked to changes in microRNA expression.[12] Since HPV18 has been associated with very aggressive adenocarcinomas, this finding may explain why Dr Gey was surprised by the prolific growth of HeLa cells in culture. Routine Papanicolaou smear screening may not detect rapidly progressive cervical carcinomas; the new HPV vaccine holds the promise of preventing these tumors.

Gey and colleagues[13] published data with HeLa cells in 1952, reporting the ''evaluation in vitro of the growth potential of normal, early intra-epithelial, and invasive carcinoma from a series of cases of cervical carcinoma.'' Only 1 strain of cervical carcinoma cells was established in ''continuous roller-tube cultures for almost a year,'' which grew in a medium of chicken plasma, bovine embryo extract, and human placental cord serum: HeLa.[13] Dr Gey's roller-tube technique for tissue culture was another significant scientific contribution and was used by John Enders, PhD, and colleagues in their work cultivating poliomyelitis virus in nonnervous system tissue.[4] Perhaps less well known in the history of poliomyelitis research is that Dr Gey successfully propagated poliomyelitis viruses in HeLa cell culture.[14]

TISSUE CULTURE CONTAMINATION

George Gey was generous with requests for HeLa cells. Since HeLa cells were a robust, immortal cell line, easily propagated over generations in culture, Dr Gey supplied samples to scientists in the United States and internationally who were interested in studying the first established human cancer cell line. HeLa cells proliferated in cultures around the world and, as the years passed, evidence accumulated that HeLa cells had contaminated other cell lines. Interspecies cross-contamination with HeLa, easier to detect than intraspecies contamination, was described in the early 1960s.[15,16]

Several years later, in 1967, intraspecies contamination of cell lines became more readily detectable through the work of Stanley Gartler, PhD.[17] He described apparent HeLa cell contamination of 19 other human cell lines by using a technique of isoenzyme analysis of glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase (PGM) electrophoretic polymorphisms; all cell lines had the same G6PD type A and PGM type 1 phenotypes.[18] The G6PD type A variant is sex-linked and found at an increased frequency in the African American population. The phosphoglucomutase gene is autosomal and has 3 common variants. The likelihood of all cell lines having these 2 isoenzyme variants was low, and several of the tested cell lines were known to be from whites. Gartler[18] noted that ''with the continued expansion of cell culture technology, it is almost certain that both interspecific and intraspecific contamination will occur.'' He hypothesized that the G6PD subtype could have changed because of multiple divisions in culture, but he later demonstrated the stability of isoenzymes in cell culture lines over time.[19]

Methods for identifying cell lines were not limited to isoenzyme phenotypes. Karyotyping and chromosome band analysis were added to the arsenal of techniques available. Chromosome band analysis involved limited

trypsin digestion of histone proteins followed by Giemsa staining[20]; controlled exposure of nucleoproteins to trypsin resulted in their partial removal and revealed Giemsa-stained bands. The technique was time-consuming but reliable in experienced hands. Thus, in the early 1970s, the state of the art for HeLa cell identification included presence of G6PD type A, lack of a Y chromosome, and identification of a specific pattern of banded-marker chromosomes[21]; these 3 three findings were thought sufficient to define a cell line as HeLa.

In 1974, 5 cell lines—reportedly of human lineage and infected with animal viruses—were sent to the United States from the Soviet Union. All of the cell lines were revealed to be HeLa in origin.[22] In a story previously detailed,[7] the realization that HeLa cells had contaminated cultures so far afield led to a reappraisal of tissue culture stocks by the American Type Culture Collection (ATCC; Manassas, Virginia) and the Cell Culture Laboratory at the Naval Biosciences Laboratory (Oakland, California).[21–27] For instance, a follow-up study to the proper identification of the Soviet Union cell lines implicated HeLa cells as contaminants of several other cell lines.[23] The ATCC found that 27 of 56 cell lines had G6PD type A variant.[24] Further analysis revealed that several of these cell lines possessed some, but not all, HeLa markers. It was hypothesized that these variations could represent somatic cell hybridization between the original cell line and the contaminating HeLa cells.

During the previous quarter century, Dr Gey's samples of HeLa cells had multiplied in laboratories throughout the world, as they were transferred from researcher to researcher and across international borders. Several hypotheses were offered for HeLa cells' remarkable growth beyond what might be expected of a very aggressive cervical adenocarcinoma. As the first human cancer cell line, and a potent cell at baseline, it had been selected to survive in culture after countless passages, cell divisions, and viral infections. In the battle for reproduction, HeLa was best selected to outcompete other cell lines and eventually overgrew other cultures it invaded. Another possible explanation was that cell lines often came from outside laboratories. Prior to their deposition in tissue culture collection banks, the cell lines had been subjected to variable laboratory techniques. Furthermore, these laboratories undoubtedly possessed other cell lines such as the ubiquitous HeLa. Since HeLa cell contamination has been reported from air droplets,[28] poor laboratory technique would suffice to rapidly contaminate other cell lines, which would then be passed on to subsequent laboratories.[24,29]

CELL CULTURE CONTROVERSY

The debate over cell culture contamination was not always harmonious.[7,30] Contaminated cell lines went far beyond HeLa cells. In one study, human breast cancer cell lines were found to have both intraspecies and interspecies contamination. Other cell lines reported to be human cells were actually derived from hamster, rat, mouse, mongoose, or mink; gibbon cells were actually human cells; horse cells were dog cells.[25] In total, 41 of 253 cell lines (16%) were not what they had been purported to be. Years of research and numerous academic careers were built on the presumed identity of various cell lines, and clarifying incorrect data required repudiating previously reported results.[31] Alternative explanations for HeLa cell contamination were offered in some instances.[32]

Unfortunately, the impact of cell culture contamination extended far beyond the relatively narrow field of cytobiology and the researchers studying cell lines. For example, radiobiologists investigating the relation of radiation doses to cell death in human kidney cells were surprised to discover that the cells they thought were derived from human kidney were actually HeLa.[26] Controversy erupted regarding the interpretation of their results: how did irradiating malignant cells translate to normal cells when evaluating cell death?[33] The debate even ensnared Jonas Salk, MD, who stated at a conference in October 1978 that he had injected study subjects, enrolled in a vaccine trial, with HeLa cells that had contaminated his cultures[7]; however, any mention of HeLa failed to find its way into his published remarks regarding the ''theoretical' possibility of transmitting a neoplasia-inducing factor.''[34]

HeLa CELLS AND CELL CULTURE CONTAMINATION TODAY

Despite the passing of nearly 50 years since the problem first surfaced of HeLa cell contamination of tissue cultures and despite the explosive advances in molecular biology, cell culture contamination remains an important issue for the scientific community.[35–38] The problem extends far beyond HeLa cells, although they remain a culprit.[38] In one study, 45 of 252 human cell lines (18%) supplied by 27 of 93 originators (29%) were contaminated.[39] Most of the contaminants were intraspecies cells, suggesting improved detection of interspecies contamination, but still concerning. New techniques, such as amplification of minisatellite-region DNA[40] and short tandem repeat profiling,[41] which are faster and more precise than older techniques such as chromosome banding, have not been widely adopted in a standardized, universal fashion. Fortunately, there was recently a call to action on preventing contaminated cell lines.[42]

CONCLUSION

Philosophically, we wonder if Henrietta Lacks has achieved a kind of corporeal immortality through her eponymous cell line. Sir William Osler, MD, delivering the Ingersoll Lectureship titled ''Science and Immortality'' at Harvard University, Boston, Massachusetts, in 1904 pondered new lessons from modern embryology and how they may impact the meaning of death. Although he obviously knew nothing of cell lines or DNA, he could marvel that ''the individual is nothing more than the transient off-shoot of a germ plasm, which has an unbroken continuity from generation to generation, from age to age . . . 'the individual organism is transient, but its embryonic substance, which produces the mortal tissues, preserves itself imperishable, everlasting, and constant.' ''[43]

It is impossible to know what Dr Osler would have thought about immortal HeLa cells. Has Henrietta Lacks' ''germ plasm'' or ''embryonic substance'' (her DNA) provided her with an unbroken, unaltered chain to the present day so that we can claim that HeLa cells are Henrietta Lacks? Or, has her DNA evolved into a new entity—*Helacyton gartleri* has been suggested[44]—after countless cell culture passages, viral infections, and other cell line contaminants? Although the question of whether or not such a new species has evolved in the cell cultures of laboratories around the world is difficult to answer, as molecular

biology continues to expand the frontiers of our knowledge at breathtaking speed, this question may need to be answered to fully comprehend both the findings of experiments performed on HeLa cells and the ethical implications of creating what may be regarded as a new organism.

References

1. Surgical Biopsy Number 92498. Baltimore, MD: Department of Pathology, The Johns Hopkins Hospital; 1951.
2. Jones HW. Record of the first physician to see Henrietta Lacks at the Johns Hopkins Hospital: history of the beginning of the HeLa cell line. *Am J Obstet Gynecol*. 1997;176:227S–228S.
3. Autopsy Protocol 23260. Baltimore, MD: Autopsy Records of The Johns Hopkins Hospital; 1951.
4. Harvey AM. Johns Hopkins—the birthplace of tissue culture: the story of Ross G. Harrison, Warren H. Lewis, and George O. Gey. *Johns Hopkins Med J Suppl*. 1976:114–123.
5. Bang FB. History of tissue culture at Johns Hopkins. *Bull Hist Med*. 1977; 51:516–537.
6. Gey GO. *Some Aspects of the Constitution and Behavior of Normal and Malignant Cells in Continuous Culture*. New York, NY: Academic Press; 1955. *The Harvey Lectures, Series L, 1954–1955*.
7. Gold M. *A Conspiracy of Cells: One Woman's Immortal Legacy and the Medical Scandal It Caused*. Albany, NY: State University of New York Press; 1986.
8. Culliton BJ. HeLa cells: contaminating cultures around the world. *Science*. 1974;184:1058–1059.
9. Jones HW, McKusick VA, Harper PS, Wuu KD. George Otto Gey: the HeLa cell and a reappraisal of its origin. *Obstet Gynecol*. 1971;38(6):945–949.
10. Schwartz PE, Hadjimichael O, Lowell DM, Merino MJ, Janerich D. Rapidly progressive cervical cancer: the Connecticut experience. *Am J Obstet Gynecol*. 1996;175:1105–1109.
11. Meissner JD. Nucleotide sequences and further characterization of human papillomavirus DNA present in the CaSki, SiHa, and HeLa cervical carcinoma cell lines. *J Gen Virol*. 1999;80:1725–1733.
12. Martinez I, Gardiner AS, Board KF, Monzon FA, Edwards RP, Khan SA. Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. *Oncogene*. 2008;27:2575–2582.
13. Gey GO, Coffman WD, Kubicek MT. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Res*. 1952; 12:264–265.
14. Scherer WF, Syverton JT, Gey GO. Studies on the propagation *in vitro* of poliomyelitis viruses. *J Exp Med*. 1953;97:695–710.
15. Defendi V, Billingham RE, Silvers WK, Moorhead P. Immunological and karyological criteria for identification of cell lines. *J Natl Cancer Inst*. 1960;25: 359–385.
16. Brand KG, Syverton JT. Results of species-specific hemagglutination tests on "transformed," nontransformed, and primary cell cultures. *J Natl Cancer Inst*. 1962;28:147–157.
17. Gartler SM. Genetic markers as tracers in cell culture. In: Westfall BB, ed. *Second Decennial Review Conference on Cell Tissue and Organ Culture*. Washington, DC: US Government Printing Office; 1967:167–195. *National Cancer Institute Monograph No. 26*.
18. Gartler SM. Apparent HeLa cell contamination of human heteroploid cell lines. *Nature*. 1968;217:750–751.
19. Auersperg N, Gartler SM. Isoenzyme stability in human heteroploid cell lines. *Exp Cell Res*. 1970;61:465–467.
20. Wang HC, Fedoroff S. Karyology of cells in culture: trypsin technique to reveal G-bands. In: Kruse PF Jr, Patterson MK Jr, eds. *Tissue Culture Methods and Applications*. New York, NY: Academic Press; 1973:782–787.
21. Nelson-Rees WA, Flandermeyer RR. HeLa cultures defined. *Science*. 1976; 191:96–98.
22. Nelson-Rees WA, Zhdanov VM, Hawthorne PK, Flandermeyer RR. HeLa-like marker chromosomes and type-A variant glucose-6-phosphate dehydrogenase isoenzyme in human cell cultures producing Mason-Pfizer monkey virus-like particles. *J Natl Cancer Inst*. 1974;53:751–757.
23. Nelson-Rees WA, Flandermeyer RR, Hawthorne PK. Banded marker chromosomes as indicators of intraspecies cellular contamination. *Science*. 1974;184: 1093–1096.
24. Lavappa KS, Macy ML, Shannon JE. Examination of ATCC stocks for HeLa marker chromosomes in human cell lines. *Nature*. 1976;259:211–213.
25. Nelson-Rees WA, Flandermeyer RR. Inter- and intraspecies contamination of human breast tumor cell lines HBC and BrCa5 and other cell cultures. *Science*. 1977;195:1343–1344.
26. Nelson-Rees WA, Flandermeyer RR, Daniels DW. T-1 cells are HeLa and not of normal human kidney origin. *Science*. 1980;209:719–720.
27. Nelson-Rees WA, Daniels DW, Flandermeyer RR. Cross-contamination of cells in culture. *Science*. 1981;212:446–452.
28. Coriell L. *Detection and Elimination of Contaminating Organisms*. Bethesda, MD: US Department of Health, Education and Welfare; 1962. *National Cancer Institute Monograph No. 7*.
29. Grimwade S. HeLa takes over. *Nature*. 1976;259:172.
30. Chatterjee R. Cases of mistaken identity. *Science*. 2007;315:928–931.
31. Nelson-Rees WA, Akeson RA, Cailleau R. Lung organ-specific antigens on cells with HeLa marker chromosomes. *J Natl Cancer Inst*. 1978;60(6):1205–1207.
32. Pathak S, Siciliano MJ, Cailleau R, Wiseman CL, Hsu TC. A human breast adenocarcinoma with chromosome and isoenzyme markers similar to those of the HeLa line. *J Natl Cancer Inst*. 1979;62(2):263–271.
33. Broad WJ. The case of the unmentioned malignancy. *Science*. 1980;210: 1229–1230.
34. Salk J. The spector of malignancy and criteria for cell lines as substrates for vaccines. In: Petricciani JC, Hopps HE, Chapple P, eds. *Cell Substrates and Their Use in the Production of Vaccines and Other Biologicals*. New York, NY: Plenum Press; 1979:107–113.
35. Stacey GN, Masters JRW, Hay RJ, Drexler HG, MacLeod RAF, Freshney RI. Cell contamination leads to inaccurate data: we must take action now. *Nature*. 2000;403:356.
36. Masters JR. HeLa cells 50 years on: the good, the bad and the ugly. *Nat Rev Cancer*. 2002;2:315–319.
37. Nardone RM. Eradication of cross-contaminated cell lines: a call for action. *Cell Biol Toxicol*. 2007;23(6):367–372.
38. Lucey DJ, Walsh MA, Costello R. Imposter cell lines. *Laryngoscope*. 2006; 116:161–162.
39. MacLeod RAF, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. *Int J Cancer*. 1999;83:555–563.
40. Silva LM, Montes de Oca H, Diniz CR, Fortes-Dias CL. Fingerprinting of cell lines by directed amplication of minisatellite-region DNA (DAMD). *Braz J Med Biol Res*. 2001;34:1405–1410.
41. Masters JR, Thomson JA, Daly-Burns B, et al. Short tandem repeat profiling provides an international reference standard for human cell lines. *PNAS*. 2001; 98(14):8012–8017.
42. Lacroix M. Persistent use of "false" cell lines. *Int J Cancer*. 2008;122:1–4.
43. Osler W. *Science and Immortality: The Ingersoll Lecture, 1904*. New York, NY: Arno Press; 1977.
44. Van Valen LM, Maiorana VC. HeLa, a new microbial species. *Evol Theor Rev*. 1991;10(1):71–74.