HeLa Cell Line [1]

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The HeLa cell line was the first immortal human cell line that George Otto Gey, Margaret Gey, and Mary Kucibek first isolated from Henrietta Lacks and developed at The Johns Hopkins Hospital [4] in Baltimore, Maryland, in 1951. An immortal human cell line is a cluster of cells that continuously multiply on their own outside of the human from which they originated. Scientists use immortal human cell lines in their research to investigate how cells function in humans [5]. Though the HeLa cell line has contributed to many advancements in biomedical research since the twentieth century, its usage in medical research has been controversial because Lacks did not consent to having her cells used for such purposes. As of 2020, scientists continue to use the HeLa cell line for numerous scientific advancements, such as the development of vaccines and the identification of many underlying disease mechanisms.

A cell line is a group of cells that multiply on their own, outside of an organism, typically in a research laboratory. Cell lines can be either immortal or finite in lifespan. Immortal cell lines replicate continuously, whereas finite cell lines have a limit to how much they can replicate. Normal human cells have finite lifespans because they have internal controls that regulate how many divisions each cell can undergo. However, some cells are immortal and do not die after a set number of divisions, as a result of alterations that can happen to certain cells, like cancer cells for example. That property of immortal cell [6] lines, like HeLa, makes them durable and prolific for scientific research.

Researchers can use human cell lines as a model to study cellular processes outside of thehumans [5] from which the cells originated. In other words, researchers can use human cell lines to study the causes of diseases within human cells without having to infect humans [5]. Because many medical researchers use laboratory-grown human cells to understand how cells work and test theories on the causes and treatments of diseases, they require that those cells are homogeneous, or the same throughout experiments, and that those cells are able to grow indefinitely. That establishes scientific reproducibility, which ensures the credibility of research. With the homogeneity and immortality of cells in cell lines like HeLa, that helps ensure that when using laboratory-grown cells, different scientists yield the same results when replicating other scientists' research using those cells. The HeLa cell line was the first immortalized cell line and researchers have used HeLa cells to achieve a diverse array of scientific discoveries. Science writer Rebecca Skloot describes the history of HeLa cells and the patient from which they came in her book, *The Immortal Life of Henrietta Lacks*.

The HeLa cell line came from a cervical tissue sample from Lacks, a patient diagnosed and treated for terminal cervical cancer at The Johns Hopkins Hospital [4] in Baltimore, Maryland, in 1951. Lacks's surgeon Howard Wilbur Jones characterized Lacks's cancer as a rare adenocarcinoma, a cancer that starts in glandular cells, which are cells that cover certain internal organs and produce and secrete various fluids into tissues. During Lacks's first surgery, Jones took a sample of Lacks's tumor cells from her cervix [7] and provided it to George Otto Gey's research laboratory at TheJohns Hopkins University [8] also in Baltimore, Maryland. According to Skloot, patients at The Johns Hopkins Hospital [4], like Lacks, routinely had their cells collected to aid in research endeavors at the hospital. Physicians often collected patients' cells without their patient's knowledge and, according to Skloot, in the early 1950s, society had not reached a common understanding of informed consent [9]. The term informed consent [9] describes the process by which healthcare professionals or researchers ensure their patient understands the purpose, benefits, risks, and alternatives of their test or treatment.

Prior to the early 1950s, researchers attempted to grow human cells in the laboratory, but those cells could not sustain themselves and multiply for more than a few weeks. Around that time, tissue culture practices differed from contemporary practices. Tissue culture is the practice of growing tissues or cells outside of the individual from which the tissues or cells originated. It necessitates the use of nutritional media, that provides the tissues with nutrients to enable their sustained survival. However, in the 1950s, there was not yet standardized equipment or nutritional media for growing cells. As such, researchers often developed their own media in the laboratory. Furthermore, researchers did not typically use sterile techniques, which have been shown to prevent contamination of biological materials.

George Otto Gey was one of the researchers whose lab aimed to develop an immortal human cell line. According to professor John R. Masters, Gey was a brilliant and highly-respected scientist, and had spent thirty years growing cells before obtained Lacks's cervical cancer specimen. In 1951, George Otto Gey was head of tissue culture research at The Johns Hopkins Hospital [4], and his wife, Margaret, was their lab's chief technician and oversaw operations within the lab. According to Johns Hopkins Medicine, George Otto Gey's goal was to develop an immortal human cell line that could form the basis for future cancer research. In the early 1950s, Gey and his wife developed their own form of nutritional media in which they sought to grow cells in a laboratory setting. Skloot describes the media mixture that the Geys created as a cocktail, as it comprised of liquid from the heart of a chicken [10], calf embryo extract, and blood from a humanumbilical cord [11]. That nutritional media was able to sustain Lacks's cancer cells and when George Otto Gey's research assistant Kubicek cultured Lacks's cancer cells, the cells continued

to multiply. George Otto Gey and Kubicek named the HeLa cell line after Lacks, with He for the first two letters of Henrietta and La for the first two of Lacks.

The HeLa cell line multiplied at an unprecedented rate, actualizing Gey's goal of creating the first immortalized human cell line. Kubicek found that the HeLa cells derived from Lacks's cervical cell sample replicated twenty times faster than Lacks's non-cancerous cells. When the HeLa cell line continued to sustain itself in the lab, Gey informed his colleagues that his lab may have grown the first immortal human cell line, as Lacks's cells continued to replicate on their own, seemingly indefinitely. Gey offered his colleagues vials of HeLa cells so they could use those cells for their research experiments.

In the mid twentieth century, researchers focused on locating other cell lines similar to HeLa. Though increased media and equipment standardization made cell culture more technically feasible and enabled researchers to grow different types of cells, other cells did not grow at the same rate as HeLa cells. Though other researchers attempted to grow immortal cell [6] lines in their own labs and seemingly successfully did so, in 1968, researcher Stanley Gartler revealed that many of those cell lines had been contaminated by HeLa cells, and that many reportedly new cell lines were just more HeLa cells. It was then discovered the HeLa cells could float through the air on dust particles and despite scientists' efforts to control contamination, HeLa cells colonized many other cell lines. In 1974, researcher Walter Nelson-Rees developed a method to authenticate cell lines to resolve the issue of mislabeled cell lines and he exposed many of the cell lines that had been contaminated by HeLa cells.

Researchers have used HeLa cells for vaccine development research, such as for the polio vaccine. In 1953, Gey showed that the polio virus could infect HeLa cells. Polio can cause paralysis among many other nervous system problems. Researcher Jonas Salk had been focusing on developing a polio vaccine. However, a large number of HeLa cells were needed to test his polio vaccine. Therefore, in 1953, Tuskegee University in Tuskegee, Alabama, established a cell culture factory to supply Salk and other labs with HeLa cells. In 1955, Salk used HeLa cells to produce the first polio vaccine. Later, biologist Harald zur Hausen used HeLa cells to identify the viral origins of a majority of cervical cancer cases. After zur Hausen connected HeLa cervical cancer cells with the human papillomavirus, or HPV, other researchers eventually developed a series of vaccines to prevent people from getting HPV. HPV is a sexually-transmitted virus that causes cervical, vaginal, anal, penile, mouth, and throat cancers.

Researchers have also used HeLa cells to study how cells normally work. In 1955, researchers used HeLa cells to study the nutritional needs of cells in tissue culture media. Findings from that experiment could inform best practices for culturing other cell lines. In 1966, researchers used HeLa cells to study RNA metabolism in the cell nucleus [12] and how RNA is located throughout the cell. RNA metabolism is now known to govern a variety of other processes, such as cell replication. In 1979, HeLa cells demonstrated that glutamine, rather than sugar, is the major energy source for cancer cells. Researchers' knowledge of the energy source for cancer cells can help researchers who grow cancer cells for their studies. In 2004, researchers used HeLa cells to perform large-scale characterization of over two-thousand regulatory phosphorylation events in the cell, which are events that mediate cellular processes, such as cell division, cell growth, and cell aging.

HeLa cell research has contributed to a better understanding of various diseases from bacterial infections to cancer. In 1984, researchers used HeLa cells to identify and reveal the mechanism by which *Escherichia coli*, or *E. coli*, bacterial strains attach to HeLa cells. Some strains of *E. coli* can cause illness, such as diarrhea, urinary tract infections, or respiratory illness. If scientists understand the mechanism by which bacterial strains attach to HeLa cells, they can research how to prevent bacterial illnesses. In 1993, researchers tested the anti-cancer chemotherapy drug Taxol in HeLa cells to validate Taxol's mechanism to halt cancer cell division. Because the drug was effective in stopping the infinite growth of HeLa cells, the researchers concluded that it may also be effective in stopping the growth of other cancer cells.

In the twenty-first century, researchers continued to question the extent at which HeLa cells contaminated other cell lines. In 2002, researcher John R. Masters published a *Nature* review article on the HeLa cell line, discussing the historical and contemporary use of HeLa cells. Despite technological advancements for cell line authentication, scientists still use mislabeled cell lines in research, many of which are in fact HeLa cells. Masters noted that HeLa cells have contaminated other cancer cell lines and cells from non-human species. In contrast to many other cell lines, the HeLa cell line has maintained its genetic authenticity. Researchers using mislabeled cell lines, whether knowingly or unwittingly, can compromise their data because their studies are no longer reproducible when the cells that they used are not identifiable. Reproducibility is necessary for experiments' scientific credibility because, if an experiment has different results each time scientists repeat it, the experiment's findings cannot be true. Mislabeled cell lines posed a threat to the integrity of scientific research experiments because if HeLa cells had contaminated what scientists reported as new cell lines, any research using those supposed cell lines would not be novel, as their research was simply on more HeLa cells.

The HeLa cell line has prompted various bioethical debates, some which are still ongoing as of 2020. In 1951, segregation was legal in Baltimore, Maryland, and according to Skloot, black people did not question white people's professional expertise, especially that of white doctors. The Johns Hopkins Hospital [4] was the only hospital accessible to Lacks, the donor of the HeLa cell line, and Lacks received her care in what Skloot refers to as the colored ward of the hospital. Lacks never gave consent for scientists to use her cells for research. However, it was common in the 1950s for physicians to collect samples from their patients for research without their patients' knowledge or consent, especially for patients receiving their care in the public wards. Skloot recounts in her book how Lacks's daughter Deborah Lacks Pullum had heard stories about The Johns Hopkins Hospital [4] enlisting black people for research and how Lacks's family was not well educated about the disease that killed Henrietta Lacks.

According to researcher Laura M. Beskow of Duke University School of Medicine in Durham, North Carolina, the HeLa story led to debates regarding informed consent [9], commercialization and compensation, patient privacy and confidentiality, race, poverty, health disparities, familial implications of genetic information, biospecimen ownership, and integrity in biomedical research. Beskow argued that Lacks and her family's story about HeLa influenced policy and regulatory changes in the United States on informed consent [9]. For example, though Gey, the original HeLa researcher, gave away HeLa cells to his colleagues and to anyone who requested them, the cell line and its resulting discoveries were extremely lucrative. However, the Lacks family received no financial compensation and continued to live in poverty.

In 1990, a similar instance involving physicians and researchers creating a cell line without their patient's knowledge or consent arose through the case Moore v. Regents of University of California, when the Regents of the University of California applied for a patent on a cell line derived from John Moore's body. In that case, the Supreme Court of California considered Lacks's case when deciding whether a person's discarded cells are their property and if those cells can be commercialized. The Court ruled that a person's discarded cells are not their personal property and that cells from their body can be commercialized.

In 2013, researchers in Heidelberg, Germany, published a detailed genomic analysis of the HeLa cell line's DNA and RNA profile, which raised discussion about genetic privacy, consent, and the legal and ethical norms encompassing genetic research. When the team of researchers, led by geneticist Lars Steinmetz, first published their article, "The Genomic and Transcriptomic Landscape of a HeLa Cell Line," as an early online version in the journal *G3: Genes, Genomics, Genetics*, they also released the gene sequencing information to online databases. That made the genetic information readily available to scientists and the public alike. In response, the Lacks family, who are genetic descendants of Henrietta Lacks and therefore, share DNA with that within the HeLa cell line, protested the publication as it was done without the family's consent. They argued that publishing that genetic data could disclose important, personal health information that might enable life insurance or disability coverage companies to unfairly discriminate against the family. Furthermore, because the cell line was called HeLa, they stated there was no possibility for privacy, as the cell line name would always connect to Lacks and her family.

After scientists proved it was possible to derive genetic information about both Henrietta Lacks and her family from the HeLa DNA sequence, the research team withdrew their publication. Shortly after the information was taken offline, *G3: Genes, Genomics, Genetics* released an editorial noting that previously published genetic sequences had not required consent. However, many others indicated that the case exemplified an internal flaw within the system of scientific cultural norms regarding consent, asserting that the notion that scientists did not historically gain consent for genome [13] sequencing was a marker that the problem was systemic. After meeting with the Lacks family members, the NIH concluded that the HeLa genome [13] information would be available for scientists on a controlled-access contingency. That meant that scientists would have to request access to the genome [13] information from a working group at the NIH, on which two members from the Lacks family would delegate. That decision enabled scientists to continue utilizing the genomic data to further promote research involving the HeLa cell line.

The HeLa cell line has enabled scientific advancements in many research areas, from the development of the polio vaccine to the understanding of how human papilloma virus can cause cervical cancer. The HeLa cell line story has also caused controversy regarding race and class, patient rights, and informed consent [9] in healthcare. In a 2013 *Nature* article, NIH director Francis Collins stated that Henrietta Lacks posthumously continues to make contributions to science, not only in regards to the scientific impact of the HeLa cell line, but also by promoting modern policy and ethical considerations that prioritize human subject protections, privacy, and respect.

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