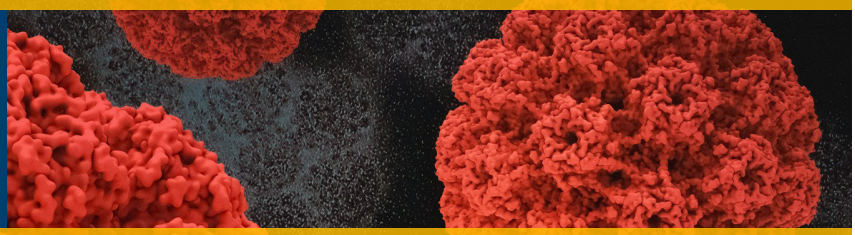
Oncovirus Exposed: The HPV/Cancer Connection



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Transformation of Original Research Article

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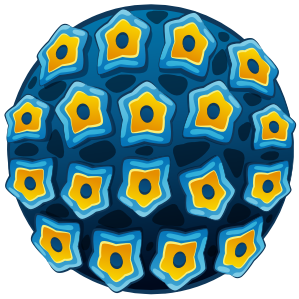
Abstract

Human papillomavirus (HPV) causes nearly all cervical cancers and many other cancers that occur throughout the body in both men and women. Although there are 488 identified HPV types, only 12 are responsible for these cancers. The sequencing of the HPV genome has led to the identification of HPV types which are more likely to cause cancer. Of these, the most carcinogenic is HPV16. In this study, HPV16 genome data is evaluated to identify contributing events that may lead to carcinogenesis, including the role of virus evolution. Understanding the HPV16 oncovirus provides insights to treating and even preventing cancers caused by HPV16.

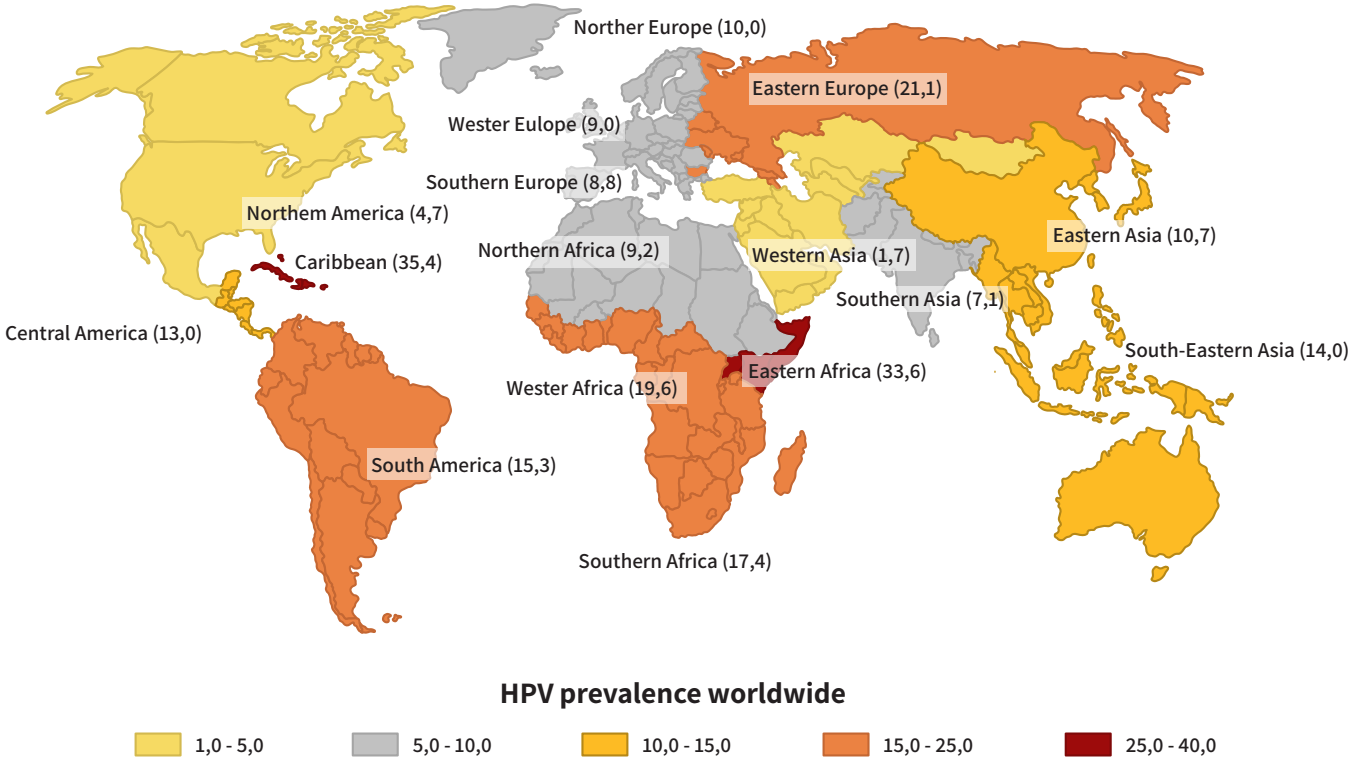


Human Papillomavirus

Background

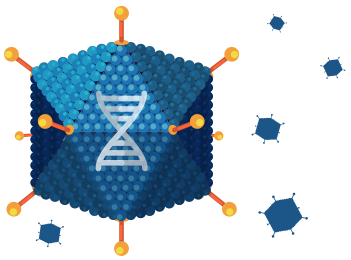
Human papillomavirus (HPV) is a common virus found in humans (Fig. 1) and one of the most prevalent ***carcinogens*** (car-SIN-oh-gens). A carcinogen stimulates the development of cancer. Carcinogens come in many forms from chemicals to radiation to bacteria to viruses. A virus that promotes the development of cancer is called an ***oncovirus*** (ON-coh-virus). There are many genotypes of HPV. The ***genotype*** (GEE-no-type) of an organism refers to the complete set of genetic material: the arrangement of DNA molecules that make up individual genes that code for various traits which are expressed as the ***phenotype*** (FEE-no-type). Most HPV genotypes do not promote the development of cancer. However, there are 12 HPV genotypes that have been identified as carcinogens. Of these 12, two are responsible for 71% of cervical cancers in females and 100% of HPV-associated cancers in males. These two HPVs are known as HPV16 and HPV18. Of these two viruses, HPV16 is associated with the most cases of cancers caused by HPV. In this metanalysis research, scientists evaluated data from multiple HPV16 studies to better understand the driving forces which would cause a virus that depends on host cells to replicate to result in cancer that could kill the host organism.

Figure 1: HPV Prevalence in Women



NOTE: HPV prevalence worldwide per 100,000 inhabitants. Data from a meta-analysis of 1,016,719 women obtained from Bruni et al. (2010).

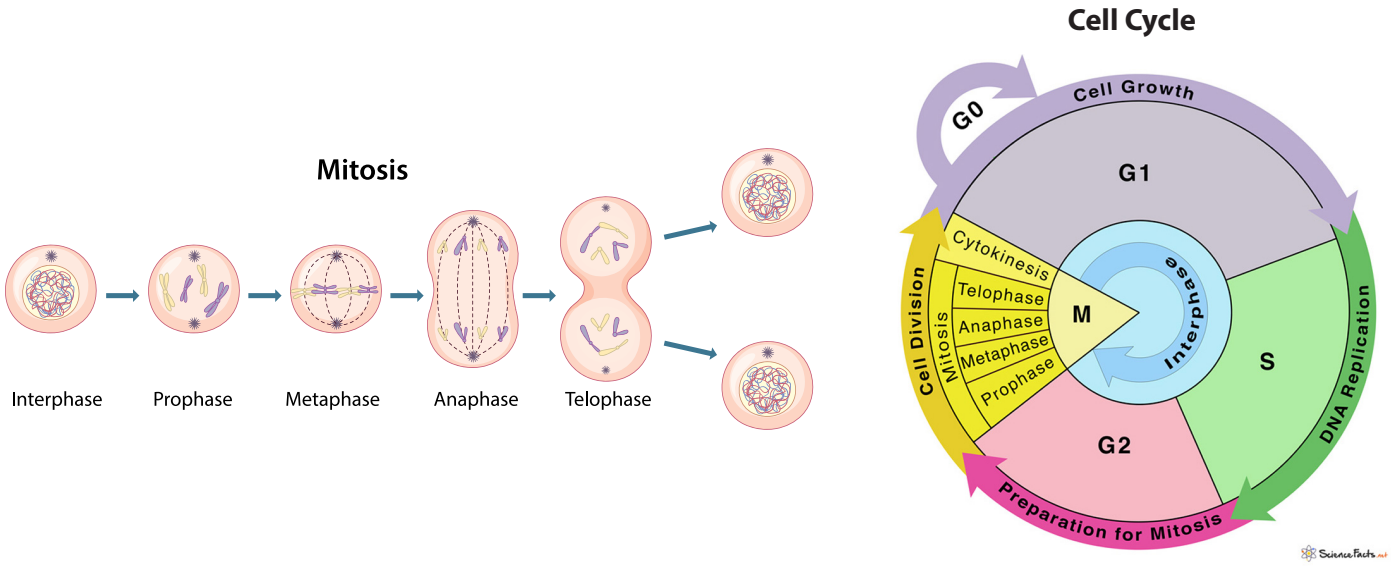
Cell Cycle



Viruses contain genetic material, sometimes a single strand of RNA or even double-stranded DNA. Although viruses contain genetic material, they lack the mechanisms required for the genetic material to replicate. Because they cannot replicate by themselves viruses are classified as non-living. HPV is an ***icosahedral*** (eye-coh-sah-HEE-drill) virus containing a double-stranded molecule consisting of the same base-pair system found in human DNA. Viruses replicate by invading a living cell and hijacking the mechanisms of the ***cell cycle*** to make copies of its own genetic material. The cell cycle is a series of events in which each cell grows, replicates its DNA, and divides into two daughter cells. The longest part of the cell cycle is ***interphase*** during which the cell grows and prepares for the next phase: ***mitosis*** (my-TOH-sis).

DNA replicates during interphase in the cell cycle, creating duplicate strands of DNA, known as ***chromosomes*** (CROW-mah-sohms). During mitosis, the duplicated chromosomes separate and migrate to opposite areas of the cell. The cell then splits in two resulting in two daughter cells with the same number of DNA strands as the original cell. After separation, each daughter cell undergoes ***differentiation***. During the differentiation process, each daughter cell becomes specialized. For example,

a heart tissue cell that undergoes mitosis will produce two daughter cells. During differentiation, each daughter cell will acquire the unique characteristics and functions of a heart cell.



HPV Genome

Considering that viruses need living cells to replicate, it is not in the best interest in the virus to kill the host. Knowing this, scientists asked why do HPV16 and HPV18 cause a disease that can kill the host? Killing the host stops the virus from replicating. This study explores the ***genomics*** (gee-NOH-mics) of HPV16, looking for genetic variants in viral DNA which may explain the high levels of cancer associated with this virus.

All DNA, including circular viral DNA, carries genetic codes. Each code is a series of three bases. Each set of three bases codes for a specific amino acid. When amino acids are linked together into a chain, a protein is formed. These proteins are necessary for optimal cellular and bodily function. Through next generation sequencing techniques (NGS), it has been determined that the DNA molecule for HPV16 consists of several regions (Fig. 2). The ***noncoding region*** (NCR) provides structural support for the DNA. Areas of the DNA that code for assembling a protein are called ***open reading frames*** (ORFs). The ORFs are identified by location. An "E" means the ORF appears early in the DNA structure and an "L" denotes ORFs that appear late in the DNA structure. Sections of the E and L areas of the ORFs have been associated with various functions and are given numerical values ranging from E1 (early 1) to E8 (early 8) and L1 (late 1) to L2 (Late 2). Sections E6 and E7 have been identified as the primary HPV oncoproteins. Specific activity at each genomic level is described in detail in Table 1.

Figure 2: HPV 16 genome diagram

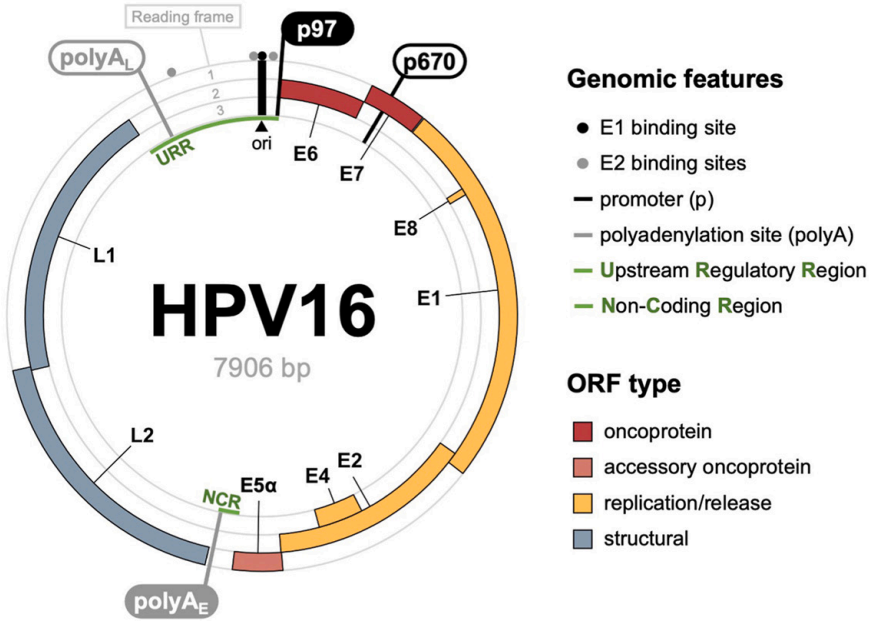


Table 1: Decoding HPV16 Genome

Expression	Genes	Functions
Early	E1	DNA helicase activity, DNA-dependent ATP binding, ATPase activity. Role in replication and replication repression.
	E2	Regulator of viral transcription and replication, control of the early expression region (early), necessary for efficient viral replication along E1.
	E4	Expressed primarily in differentiating epithelium, associated with the keratin cytoskeleton of epithelial cells in culture. Role in virus release.
	E5	HPV16 transformation activity in vitro. It possibly stimulates the initiation of cellular prevention in vivo but may have a role in the initiation of carcinogenesis.
	E6	Role in the transformation process together E7. Transcriptional activation properties. High-risk HPV's E6 inactivates p53 through rapid degradation through the ubiquitin pathway. Together with E7, it provides a cellular environment for viral replication.
	E7	Induces DNA synthesis in resting cells. E7 binds to the hypo-phosphorylated form of the pRb protein, resulting in its functional inactivation allowing functional progression to the S phase of the cell cycle. Low-risk HPV types 6 and 11's E7 protein binds less efficiently than the high-risk HPV types 16 and 18's E7 protein.
Late	L1	Larger capsid protein. Encodes main capsid protein.
	L2	Smaller capsid protein. Encodes secondary capsid protein.

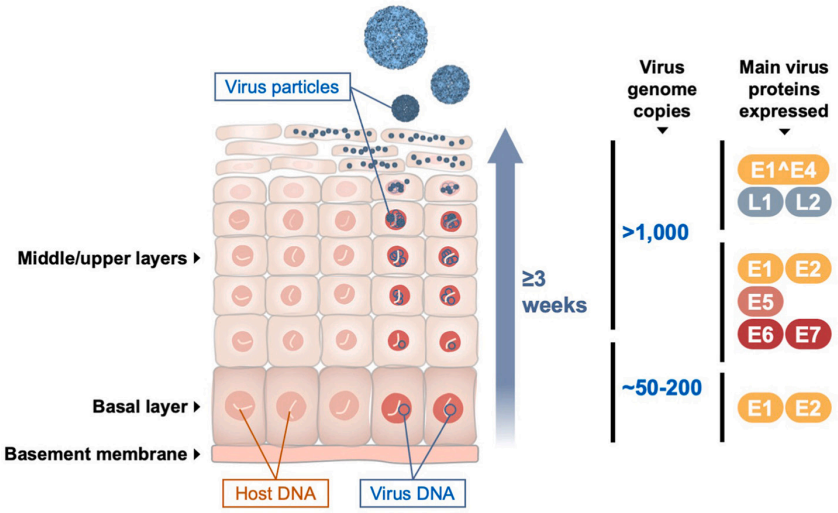
HPV Infection

Skin is a multilayered organ with upper, middle, and basal layers. Cells in the upper layer continuously shed and are replaced as cells from the middle layer mature and move up to the surface. It can take up to three weeks for a basal cell to differentiate and migrate to the middle and upper layers of skin. The HPV16 particles rest on the surface of the upper most epithelial layers, where the epithelial cells create a protective layer, preventing HPV16 particles from entering lower skin layers. As skin cells migrate to the surface, the nucleus "destabilizes", losing the ability to replicate as the skin cell dies and is sloughed off. Without a functioning nucleus in the host cell, HPV16 cannot replicate.

However, certain areas of the body have epithelial tissue that experience microtears. It is through these microtears that HPV16 can reach the basal layer of epithelial cells. Once in basal layer, the HPV16 enters the basal cell nucleus and replicates (Fig. 2).

Once inside a basal cell, the HPV16 DNA enters the nucleus and replicates, forming ***episomes*** (EP-ah-sohms). Episomes are extrachromosomal circular plasmids, which means they are circular structures of the HPV16 DNA that replicate without integrating or becoming part of the cell's DNA. Occasionally the plasmid will become part of the cell's DNA. Throughout this process, the HPV16 does not cause the cell to die. There is no inflammation to alert the body's immune system to the virus. The basal cell continues to mature and migrates to the surface, carrying it's load of HPV16.

Figure 3: Life cycle of carcinogenic HPV16 in stratified squamous epithelia.



NOTE: Microtear exposes basal cell layer where HPV16 enters the basal cell nucleus and begins replicating. Over approximately 3 weeks, the HPV16 host nucleus generates genome copies. By the time the cell matures and reaches the surface, it has produced over 1,000 copies of the infectious HPV16 genome.

Under normal circumstances, HPV16 only infects a cell and does not migrate into neighboring cells. It stays within the original host cell. However, viruses are prone to mutations, specifically deletion mutations. In a deletion mutation a section of the viral DNA is removed. DNA carries the code for making proteins. These proteins carry out specific functions for the cell and/or organism. Removing any DNA will change the code sequence, and a protein may be eliminated entirely or replaced by a different protein.

RESULTS

An Oncovirus Emerges

HPV viruses use host ***polymerases*** (poh-LIM-er-aces) to replicate. These polymerases are enzymes which turn codes on the host cell's DNA "on" and "off". This process normally takes place before a basal cell undergoes differentiation. But for HPV16 to replicate, it needs to use the host cell's polymerases during, not before, basal cell differentiation. The virus causes an overlap between these cellular functions. This overlap and conflicting signals by polymerases increase the likelihood of mutations in the host cell's DNA, increasing the chance of forming cancer.

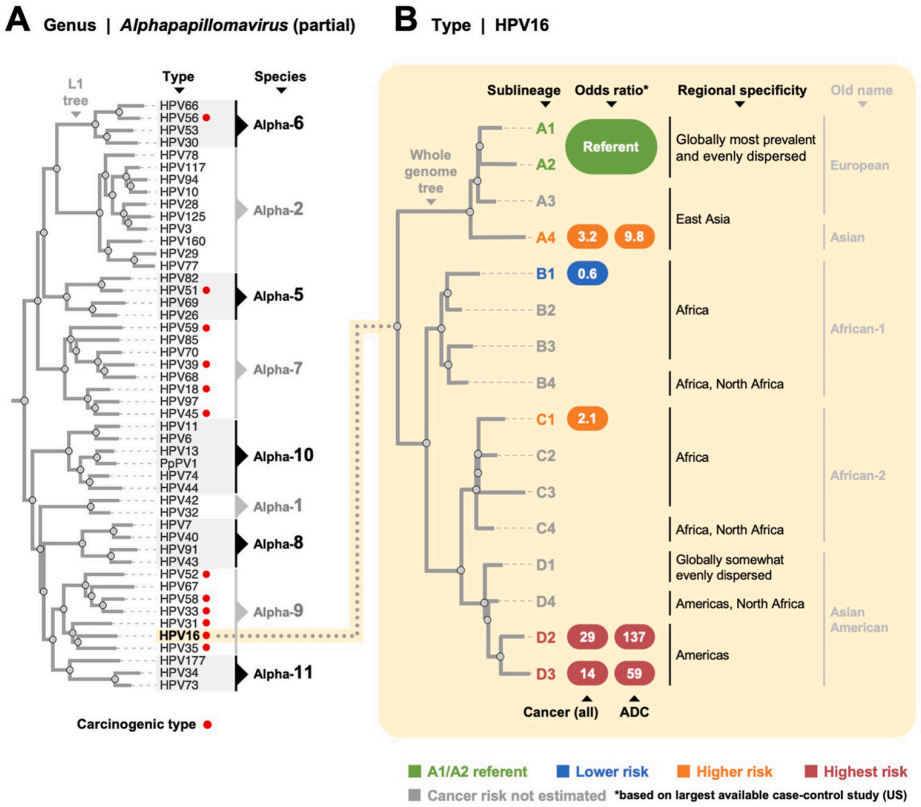
Because HPV16 uses the host cell's polymerases to replicate, the virus does not have a high mutation rate. When compared to RNA viruses that contain a single strand of genetic material, the HPV mutation rate is 1000 times lower than RNA viruses. But when compared to human mutation rates, HPV mutation rates are 500 times higher than humans.

Oncovirus Evolution

Using a genome tree, the evolution of different types of HPV were studied and classified by shared properties. These categories are called "species". Although divided into species, remember viruses are not alive. There are 12 separate genera of HPVs: Alpha- Beta-, Gamma, Mu-, and Nu-***papillomavirus*** (pap-ah-LOW-ma-vy-rus). The genus alpha-papillomavirus contains all the carcinogenic HPV types. Of the 13 species of HPV in this genus, 12 are classified as oncoviruses.

Although mutations within the host cell may account for the formation of cancer, scientists sought out other contributing factors which could cause a virus to trigger cancer, especially when it depends on the host to replicate. Using a genome tree for HPV, scientists investigated a possible association between virus evolution and the incident of cancer (Fig. 4). Of the seven HPV strains in species studied in Alpha-9, six are known oncoviruses, including HPV16 (Fig. 4A). Focusing on HPV16, multiple sub-lineages of the virus were identified (Fig. 4B). Strains of HPV16 are found across the globe, yet the risk of developing cancer from an HPV16 strain varies with location and level of evolution. Within HPV16 sub-lineage, the cancer risk for the least evolved strains, B and C appear to have lower cancer risk, while the strains that are most evolved, sub-lineages A4, C1, D2 and D3, are the most recently evolved and carry higher incidents of cancer.

Figure 4: Evolutionary Relationships of HPV16



Conclusion

The development of HPV16-induced cancers likely involves numerous contributing events from transmission to microtears to mutations to virus evolution. Deciphering and analyzing the entire genome of the oncovirus HPV16 has led to increased understanding of the mechanisms which contribute to cancer development. With increased understanding of how HPV16 leads to cancer, more effective therapeutics and clinical protocols regarding vaccines can be developed. As with all well-thought-out science investigations, more research questions are raised. Continued study of the mechanisms responsible for the morbidity and mortality rates associated with HPV-driven cancers are needed.