I am Karen Adler Storths. I’m a Professor Emerita in the Department of Diagnostic and Biomedical Sciences at The University of Texas School of Dentistry in Houston. I’ve studied human papillomavirus and its role in oropharyngeal and cervical cancer for my entire career of 31 years. Today I will be covering the basic biology of human papillomavirus infection in order to prepare you for the additional lectures that will occur in this series.

Upon completion of this lecture you’ll be able to discuss the diversity of HPV types and the diseases caused by HPV. You will understand the function of the HPV genes and be able to discuss the replication cycle of HPV especially how HPV contributes to cancer development.

We will begin with the general features of human papillomavirus. It is what we call a naked capsid virus meaning it doesn’t have an envelope such as is found in influenza virus. It’s one of the smaller viruses being only 55 nanometers in diameter. It has a small double-stranded circular DNA genome which contains eight genes and a regulatory region. There are over 150 genotypes of HPV that have been defined by DNA homology as opposed to serology. With most viruses we classify them according to serotype but with HPV the structural proteins of the virus are so similar that it requires us to compare their genomes in order to classify them. The cellular target of HPV is the epithelial cell or the keratinocyte of the skin or the mucous membranes. Papillomaviruses induce cell proliferation, thus, tumor formation. Those tumors may be benign as is in the case of common warts or malignant occurring along with the presence of other co-factors such as occurs in cancer of the cervix and the oropharynx.

I’d like to begin with a brief history of the papillomaviruses. Even though HPV became a major player in the world of carcinogenesis in the early 70s we have known about these viruses and their transmissibility since the early 1900s. In 1907 it was shown that an extract from a common wart could transfer disease. In other words the cells of the wart were ground up and that mixture of a virus and cellular proteins was applied to the skin and other warts formed on naïve skin. In 1935 studying the cottontail rabbit papillomavirus it was shown that this virus could cause skin cancer in the rabbit. In 1949 it was shown that genital warts were infectious similar to what was shown with the common wart extract and had viral particles because at that time we had the advent of electronmicroscopy and we could see the viral particles. In 1959 bovine papillomavirus was shown to be able to transform rodent cell lines in culture. In other words, normal cells became more transformed towards a malignant phenotype when they were infected with bovine papillomavirus. In 1972 it was shown that an extract from a lesion in a patient who had epidermodysplasia verruciformis which causes immunosuppression could form --- could cause warts on naïve areas of the skin. The big homerun came in the early 1970s when human papillomavirus was proposed as the etiologic agent of cervical cancer and since the mid-70s HPV has really been a tremendous topic of research. In the early 80s studies were done and showed that HPV 16 and 18 were actually found in biopsies of cervical cancer and in cell lines derived from cervical cancers. That work continued for a very long time and continues to this day and in 2006 the FDA approved a quadrivalent
vaccine for two of the low-risk HPV types and two of the high-risk HPV types which we will talk more about in a few minutes. And lastly in 2008 Harold zur Hausen who is pioneer of HPV research was awarded the Nobel Prize for his studies showing the association of HPV with cervical cancer.

There are five evolutionary groups of HPV with different epithelial tropisms and disease associations. In recent years it has become clear that the alpha and beta papillomaviruses cause only asymptomatic infections in immunocompetent individuals. This is because the viruses are well-adapted to the host and can be maintained in the population without causing apparent disease. The alpha papillomaviruses include most of the viruses that we'll be talking about today. The alpha papillomaviruses include the low-risk mucosal types that cause genital warts and the high-risk mucosal types that are associated with cervical and oropharyngeal cancer. The beta papillomaviruses include those types that are capable of inducing skin cancer in immunocompromised individuals. And as I mentioned before this was studied first in patients with epidermodysplasia verruciformis or EV and one of the classic features of this disease is immunosuppression. And these lesions tend to occur on sun exposed areas of skin thereby implying the necessity of not only the virus but other co-factors in tumor development.

So for the purposes of most of the remainder of my talk I'll talk about the prototype human papillomaviruses. In terms of mucosal infections HPV 6 and HPV 11 are considered to have a low risk for malignant conversion. Very rarely are these viral types found in any type of malignant condition. HPV 16 and HPV 18 are the prototype high-risk types meaning they are found in the majority of cases of cancer associated with HPV. In terms of cutaneous infections the common types are HPV 1, 2, and 4 and there are numerous types in EV patients. Some have a low risk for malignant conversion and others who have a high risk for malignant conversion specifically HPV 5 and HPV 8.

In order to understand HPV replication and its role in carcinogenesis it's essential to be familiar with the viral genes and their function starting with this area called the URR. This is the upstream regulatory region. It contains genetic elements that regulate gene expression and thus regulate replication of the virus. L1 and L2 are the viral structural proteins and they are very similar amongst all human papillomaviruses. E1 and E2 are involved in DNA replication and E2 is also involved in regulation of gene expression. E4 is a membrane signaling protein that is involved in release of virus from infected cells. E5 is also a membrane signaling protein that may play a role in carcinogenesis. Some recent work has shown that it may play an early role in this process. E6 and E7 are the major viral oncoproteins. E6 targets the p53 protein. P53 is called the guardian of the genome. That is the major player in cells that signals cells that have genetic damage to undergo apoptosis or programmed cell death. If it is not in the picture because HPV E6 targets it for degradation then cells that have genetic damage can continue to replicate. E6 is also involved in increasing expression of telomerase. Telomerase is a multisubunit enzyme that is involved in maintaining the ends or the telomeres of the
chromosomes. Normally as cells age chromosomes shorten but in the case of overexpression of telomerase the chromosomes maintain their length and the cells can continue to divide. E7 targets the active form of the retinoblastoma tumor suppressor product. This protein, the phosphorylated form called pRB, functions in the G1 phase of the cell cycle and controls entry of cells into the S phase which is the DNA replication phase. If the retinoblastoma virus -- protein is inactivated then cells can progress into the S phase of the cell cycle with no checkpoint and continue to replicate.

This slide shows how HPV genes are expressed in viral replication. The virus first infects cells of the basal layer of the epithelium through some type of small cut or microabrasion. We get an early phase of genome amplification where you see the viral genome being maintained in its circular structure or we call this an episome and it replicates up to about 200 copies per cell and then it continues to be replicated in the cell at low numbers. As you move towards the outer layers of the epithelium where the cells are more differentiated you get expression of the genes that control DNA replication, gene expression, and E6 and E7 as they contribute to cell growth. Then you begin to get expression of the late proteins of the virus which are L1 and L2, the structural proteins of the virus as you get the suprabasal layer and the granulocell --- layer and then the viruses are assembled and then they are released as cells from the cornified layer exfoliate. So this is not a lytic viral infection. The cells are --- The virus is actually released in cells that are shed from the skin or mucous membranes. Although there are many similarities in genome organization of the human papillomaviruses there are differences in protein function and expression patterns that contribute to the many different disease presentations of HPV.

So let’s take a look at some clinical pictures of common benign HPV-induced cutaneous lesions. In Figure A you see common hand warts which occur on the fingers, they can occur on the palms and in the nail bed and these are most commonly caused by HPV 2, HPV 7, and HPV 57. In Figure B you see plantar warts that occur on the bottom of the foot. These are most commonly associated with HPV 1, 2, 4, and 63. In Figure C you see what are called flat warts on the forehead. These are most commonly associated with HPV 3 and HPV 10. These lesions arise in different places based on differences -- minor differences in the function of proteins as we discussed in the previous slide. Most of these lesions will regress due to the immune response to the virus but others may require treatment mostly for cosmetic reasons. However, plantar warts can be very painful and usually require excision.

Here are some clinical photos of benign HPV-induced mucosal lesions or lesions of wet tissue as opposed to dry cutaneous epithelium. In Figure A we see laryngeal papillomas. These papillomas have the potential to obstruct the airway. They are caused by --- most commonly by HPV 6 and HPV 11. Although rare laryngeal papillomas or respiratory papillomatosis as it is called can occur in children who are born through an infected birth canal and they can cause major problems in these children. Figure B shows genital warts clinically called condyloma acuminata also caused almost exclusively by HPV 6 and HPV 11. And Figure C shows some oral warts
on the inside of the lip. These are basically oral condyloma acuminata and they too are caused by HPV 6 and HPV 11.

Moving on to the HPV-associated cancers. HPV has been associated with a variety of anogenital cancers. Most of the work has been done on cancers of the cervix but there is considerable literature supporting the role of HPV in other anogenital cancers. HPV has been associated with oropharyngeal cancers, that is those in the back of the oral cavity, at the base of the tongue, and in the tonsils, and as I mentioned before in non-melanoma skin cancers in patients who are immunosuppressed particularly those who have epidermodysplasia verruciformis. There are 12 human papillomaviruses that are defined by the World Health Organization as being high-risk cancer-causing types. They are 16, 18, 31, 33, 45, 35, 51, 52, 56, 58, and 59. Type 68 and 73 are likely to be included in that list soon. Prior to the advent of the HPV vaccine, the quadrivalent vaccine, 50% of cervical cancers were associated with HPV 16 and about 20% of cancers were associated with HPV 18. Other carcinogenic types such as those in the list I previously mentioned are becoming more prevalent leading to the recent FDA approval of a nonavalent vaccine that is nine HPV types and that vaccine was approved in December of 2014.

HPV is transmitted by close contact so we generally think of it as a sexually transmitted disease although individuals who have hand warts can certainly transmit them from hand-to-hand and things like that but we really think mostly of HPV these days as a sexually transmitted disease. As I mentioned the gene present the disease presentation of the virus will depend on the genotype of the virus and there are specific disease presentations that are non-malignant as well as those that are malignant. The benign lesions or warts as we call them often resolve spontaneously. The most common sexually transmitted virus in the United States is human papillomavirus. It used to be herpes simplex virus. It's estimated that at least 50% of sexually active active, non-vaccinated people will have genital HPV infections during their lifetime and most will clear without any symptoms. There is estimated to be about six million new infections per year in the United States so this virus is far from being controlled and 1% of sexually active dis adults in the United States have genital warts at any one time. As we'll see in a few minutes in the case of HPV and its association with cancer the HPV DNA that persists as an episome in benign lesions becomes integrated into the host cell DNA in HPV-associated cancers.

This slide shows how HPV contributes to cancer development and basically reiterates what I said when I talked about the function of the viral genes. The virus enters the skin through the side of a small cut or microabrasion. It is endocytosed into the cell and then it is trafficked to the nucleus. Because it is a DNA virus it replicates in the nucleus. In the case of cancer development the viral genome becomes integrated into the host cell chromosome such is such that the genes that control viral gene expression the DNA that controls viral gene expression is al not integrated and so that we get it unregulated expression of the viral oncoproteins. We have the E6 protein as I mentioned that targets p53 for degradation and also causes the an increase in telomerase so we have an
inhibition of apoptosis. We also have an interruption in the cell cycle checkpoint due to the fact that the E7 protein of human papillomavirus binds to the retinoblastoma tumor suppressor product and allows cells to progress through the cell cycle. The end result of all of that is cellular transformation that can lead to tumor development. It’s interesting to note that studies have been done on the exact sequence of amino acids in the E6 and E7 proteins of the low-risk versus the high-risk HPV types and there are significant differences in the amino acids in the E6 and E7 targeting regions of those two types.

So to summarize the steps in malignant transformation associated with HPV infection, first you have to have an infection with high-risk HPV. You get high numbers of viral episomes. Eventually HPV becomes integrated into the host cell genome. You lose the episomes. You get initiation of the S phase of cell cycling due to deregulation of cell cycling by the interaction of pRB with E7.

You get inhibition of apoptosis via p53 degradation so that cells with genetic damage can continue to replicate. There’s also deregulation of DNA repair. Cells become immortalized. The --- Their telomeres are main --- maintained so they can continue to replicate. The genomes become very un --- unstable because of this and in the presence of other cofactors you get progression towards malignancy. Some of those co-factors may be smoking, exposure to UV light, exposure to other carcinogens. But remember that most of these infections do not progress to cancer. So the million dollar or even billion dollar question still remains: What is it about the small percentage of individuals who are infected with high-risk HPV pa --- types that go on to develop cervical cancer or oropharyngeal cancer? What drives that carcinogenic process? It’s been proposed that it might be polymorphisms in DNA repair genes or polymorphisms in immune response genes. But we still do not have an answer to that very important question.

So if we look at the role of HPV in progression of cervical cancer and I --- I limit my illustration to cervical cancer here because we don’t have such a model in oropharyngeal cancer and the reasons for that will probably come up in subsequent lectures in this series. So HPV infects the cells at the basal layer and you get a small lesion and sometimes you get pro --- productive viral infection which allows the --- the virus to be spread through sexual activity. Then you --- if the virus becomes integrated into the host cell genome which occurs somewhere around this blue and pinkish grey border we --- these lesions --- this area of the spectrum is called low-grade squamous inc --- intraepithelial lesions. You begin to get integration of the viral genome and you get overexpression of the E6 and E7 oncoproteins of the virus. But as you can see here in this graphic the epithelium becomes more and more disorganized. You may still have in the top layer some shel --- some cells that are shedding virus but in the lower layer --- la --- layers of the epithelium the viruses become integrated and --- mostly integrated and is driving the cell towards true malignant conversion. In the higher grade lesions as occurs from about here on you get very much disruption of normal cell function and normal cell growth and you can have the formation of carcinoma in situ and invasive
cancer. So the accumulation of E6 and E7 in these cells predisposes them to further genetic changes that drive them from this phenotype to this invasive cancer phenotype.

So in summary there are 150 genotypes of HPV that have been cloned from clinical lesions and remember they are called genotypes because they cannot be serotyped due to the similarity of their structural proteins. The target cell is cutaneous or mucosal epithelium. We talked about the --- the low-risk prototype viruses, HPV 6 and 11, associated with genital warts and oral warts and recurrent respiratory papillomas. We talked about HPV 16 and 18 which are the prototype high-risk types that are associated with 70% of cervical cancers and we talked about the fact that malignancy is an uncommon consequence of infection. And we talked about the role of the E6 and E7 proteins in disregulating the cell cycle by molecular interactions with two very important cellular proteins, p53 and the retinoblastoma tumor suppressor product.

That concludes my presentation. We welcome your feedback.