The 'Trojan horse' oncogenic strategy of HPVs in childhood

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Review
Future Virology

HPVs are considered to be the principal cause of cervical cancer worldwide. During the last decade, their possible oncogenic involvement has also been proposed in a substantial proportion of nongenital cancers, such as breast and lung cancer. The presence of high-risk HPVs in the neonatal oral mucosa supports the transmission of HPVs from the mother to her newborn. This review presents current evidence that supports the perinatal transmission of high-risk HPVs and suggests that this may be the initial step of the oncogenic strategy of high-risk HPVs in humans. The hypothesis that children are a unique reservoir of silent high-risk HPVs, analogously to the Trojan horse, should be investigated further.

HPVs are dsDNA viruses that comprise a remarkably heterogeneous family of more than 130 types targeting the human epithelium [1]. HPVs can establish a persistent infection of the genital mucosa that can lead to the development of cervical squamous intraepithelial lesions (SILs) and eventually to invasive cervical cancer. Cervical cancer screening, primarily with the Pap smear, has reduced the incidence of this cancer in industrialized countries [2]. However, cervical cancer remains one of the most common causes of death from cancer in women, with a worldwide prevalence of 2.3 million women and 82% of new cervical cancer cases occurring in developing countries [3]. However, even in Europe, the USA and Canada, where the national health systems provide women with routine cervical screening, approximately 30,000 women die of cervical cancers each year [3]. High-risk types of HPV, such as 16 and 18, are the most significant causative agents in cervical cancinogenesis, as HPV positivity in cervical carcinoma has been documented to be 99.7% [4]. The most common high-risk HPVs detected in invasive and *in situ* cancers are HPV16, HPV18, HPV31, HPV33 and HPV45 [3,4].

To date, it is well established that the HPV family harbors important human carcinogens, causing not only the vast majority of cervical cancers, but also a substantial proportion of other types of cancers [1,5]. During the last two decades, several studies have examined the presence of HPVs in esophageal, laryngeal, oropharyngeal, lung, urothelial, breast and colon cancers and have proposed the possible involvement of HPV DNA in nongenital cancers [5]. In addition, specific types have been linked to certain cutaneous cancers [1]. However, the exact role of HPVs in the pathogenesis of these cancers has yet to be proven.

Recently, several countries have introduced two vaccines against high-risk HPVs to their vaccination programs [6]. The vaccines are bioengineered component vaccines comprising virus-like particles produced from the surface proteins of HPV16 and 18 for the bivalent vaccine and HPV16, 18, 11 and 6 for the quadrivalent vaccine. Both vaccines target high-risk HPV16 and 18 that are involved in high-grade SILs and invasive cervical cancer. The association between HPVs and the progression of cervical carcinoma provides established evidence of the expected protection of the vaccine against cervical cancer.

Detection & clinical impact of HPVs in childhood

HPVs in childhood

HPVs are pathogens that are frequently associated with a wide range of cutaneous and mucosal infections in both boys and girls [7–9]. Different HPV types can cause common warts, genital warts, low-grade – as well as high-grade – SILs and anogenital warts. Evaluation of children with anogenital warts for the possibility of sexual abuse should be considered in all cases, highlighting important legal and clinical issues [7]. HPV infection may be 'silent' and exist asymptomatically or may induce the formation of benign or malignant tumors in the genital, oral or conjunctival mucosa. Although most infections are self-sustained by the immune system and clear spontaneously, those that persist result in substantial morbidity and invoke high costs associated with the treatment of clinically relevant lesions [8,9].

Current evidence supports the notion that HPVs can be transmitted both sexually and nonsexually [10]. Based on the recent meta-analysis

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by Syrjänen, HPV vertical transmission occurs in approximately 20% of cases [10]. HPV infections in the oral mucosa of infants are silent infections and are found in less than 10% of infants [7].

HPVs in recurrent respiratory papillomatosis

Recurrent respiratory papillomatosis (RRP) is a rare condition characterized by recurrent development of benign papillomata in the upper respiratory tract [11,12]. RRP is a devastating disease in which papillomata in the upper airway cause hoarseness and difficulty breathing. The papillomata can occur throughout the respiratory tract but occur most frequently in the larynx, affecting both children and adults. Despite its benign nature, RRP may significantly affect morbidity and mortality because of its tendency for malignant transformation [13]. Depending on whether the presentation occurs before or after 12 years of age, RRP is categorized into juvenile- and adult-onset variants, respectively. The prevalence of this disease is dependent on various factors such as the age at presentation, country and socioeconomic status of the population, but is generally accepted to range between one and four per 100,000 individuals [14]. Despite the low prevalence, the economic burden of RRP is high, given the multiple therapeutic procedures required by patients [14]. It is now generally accepted that the most likely route of transmission of HPVs in RRP is from a HPV-positive mother to her child during labor [13,14]. Although cesarean section may decrease exposure of children to the HPV during childbirth, its effectiveness in preventing RRP is debatable [13–16].

RRP is most frequently caused by HPV6 and 11 [15]. Other HPVs, such as HPV13, 39, 40 and 56, have also been detected in cases of RRP [16]. During the last decade, the presence of HPV11 as a risk factor for malignant transformation of RRP has become more evident [17,18]. HPV6 may also contribute an equally important role in RRP carcinogenesis [19]. Several studies have been published that have suggested that HPV11 is associated with a more aggressive clinical course [11–20]. However, Buchinsky *et al.* recently found that the clinical course of RRP was more closely related to the age of the patient at diagnosis and at the time of the current surgery than with HPV type [21]. HPV11 is more closely related to a younger age at diagnosis than it is associated with an aggressive clinical course [21]. HPV6 and 11 exist in numerous subtypes with different activities *in vitro* [20]. Moreover, RRP tissue may contain more than one subtype or even be coinfected with other viruses that may influence the clinical outcome.

In the prospective multicenter study by Gerein *et al.*, mothers of children with RRP exhibited cytological and histological evidence of HPV infection in their genital tract, including the presence of koilocytes, koilocytotic dysplasia and condylomata acuminata [22]. The incidence of lung involvement in RRP has been estimated at 3.3%, while the incidence of cancer in patients with lung involvement was 16% [23]. Well-designed randomized controlled trials and prospective cohort studies are warranted in order to improve our understanding regarding the mechanisms underlying the development of lung involvement in RRP, the risks associated with different HPV types and subtypes, the efficacy of novel therapeutic approaches and the risk of progression to cancer.

Evidence of HPV perinatal transmission

HPVs in neonatal oral mucosa

In contrast to the consistent epidemiologic evidence of the role of sexual transmission of HPVs in adults, perinatal transmission may be principally related to HPV infection in infants. To date, the presence of HPVs in newborn oral mucosa has been confirmed [24–33]. Recently, in a study by Tai *et al.* of 108 specimens collected from neonates via endotracheal aspiration, HPV DNA was detected in 7.4% of specimens [24], while in a retrospective study by Martinelli *et al.* HPV DNA was detected in 14.1% of oropharyngeal swabs collected from 177 newborns aged 0–6 months [25].

Mother-to-infant HPV transmission

The mother seems to be the main transmitter of HPV infection to her newborn [24]. Several researchers have evaluated this concordance based on a broad spectrum of HPVs in oral and genital specimens of mothers and their recently born infants [26–33]. In a study by Park *et al.* of 291 pregnant women at over 36 weeks of gestation, HPV DNA was detected in 18.9% of pregnant women and in 3.4% of neonates [26]. In this study, the rate of vertical transmission of HPV DNA from the HPV-infected mother to her neonate was estimated at 18.2%, which was increased when the infant was delivered through an infected cervix. In a study by Smith *et al.*, HPV DNA was detected in 30% of mothers and in 1.5% of newborns, with a maternal/newborn concordance rate of 71% [27].

In a study by Rombaldi *et al.* of 63 mother–newborn pairs, HPV DNA was detected in

49 pregnant women and in 22.4% of their newborns [28]. In a study by Koskimaa *et al.* of 329 pregnant women, HPV DNA was detected in 17.9% of oral samples from newborns and in 16.4% of the cervical samples of the mothers [29]. In a prospective cohort study by Castellsagué *et al.* including 66 HPV-positive and 77 HPV-negative pregnant women and their offspring, children of mothers who were HPV-positive at the postpartum visit were approximately five-times more likely to test positive for HPV than children of corresponding HPV-negative mothers [30]. These findings were verified by a systematic quantitative review of prospective cohort studies performed by Medeiros *et al.*, who included 2111 pregnant women and 2113 newborns and showed that a positive HPV test in the mother increased the risk of vertical HPV transmission [31]. Infants born through vaginal delivery were at higher risk of exposure to HPVs than after cesarean section [31]. However, the presence of HPVs in the oral cavity of children delivered by other cesarean section or normal vaginal delivery means we cannot exclude HPV perinatal transmission via cesarean section [32].

HPV persistence in infants is a rare event [30]. Interestingly, in the study by Koskimaa *et al.*, at delivery, mother–newborn pairs had similar HPV genotype profiles, but this concordance disappeared within 2 months [29]. Similar to these findings, in the study by Park *et al.*, the neonatal HPVs found at birth were all cleared at 6 months after delivery [26]. Despite the absence of persistent infection in infants at 6 months after delivery, suggesting temporary inoculation, the true impact of the silent infection of HPVs in childhood remains to be further investigated.

Father-to-infant HPV transmission

To date, several studies have detected the presence of HPVs in human sperm cells collected from sexually active males with and without risk factors for HPV infection [34–38]. Interestingly, it has been proposed that HPVs affect sperm motility parameters and increase the incidence of asthenozoospermia [34–36]. Recently, the presence of HPVs in the male partners of infertile couples has been associated with a higher pregnancy loss rate [37]. However, these studies support the role of HPVs in human reproduction and infertility, but not in father-to-infant transmission. The hypothesis that HPV-infected sperm could be able to penetrate and infect the oocyte needs further investigation before it can be confirmed or refuted.

The role of the placenta

It has been concluded that besides the transmission route of the genital tract, there may also be transplacental transmission of HPVs *in utero* [33]. To date, several studies have demonstrated the presence of HPV DNA, including HPV16, 6, 83 and 39, in the placental tissue of pregnant women [28,29,39–42]. Moreover, HPV concordance between placental and newborn samples has been suggested [28,41]. In a study by Sarkola *et al.* of 315 mothers and 311 neonates included in the Finnish HPV Family Study, HPV DNA was detected in 4.2% of placental trophoblasts and in 3.5% of umbilical cord blood samples [41]. In a study by Rombaldi *et al.* of 49 HPV DNA-positive pregnant women at delivery, 24.5% of placentas had a positive result for HPV DNA, while in 16.3% of cases, there was type-specific HPV concordance between mother and newborn samples [28]. It has been suggested that HPV infection of the placenta could occur early in pregnancy [40].

The presence of HPV DNA in the placenta has not been related to the type of delivery in childbirth [39,41]. Recently, Gomez *et al.* have proposed that HPV infection of extravillous trophoblast cells reduces cell invasion and is associated with adverse reproductive outcomes attributable to placental dysfunction, including spontaneous preterm delivery [42]. The exact relationship between placental maternal HPV infection and neonatal prematurity remains to be elucidated [43].

The presence of HPV DNA in amniotic fluid, cord blood and placental trophoblastic cells increases the risk of oral HPVs in neonates [41]. This evidence suggests that HPVs can cross the placenta, resulting in *in utero* transmission. Other researchers have failed to detect HPVs in amniotic fluid from women with intact amniotic membranes or in the placenta, indicating transplacental transmission as a possible, but not definitive, route of HPV transmission [44,45].

HPVs in human breast milk

Infection of HPVs in maternal human breast milk and colostrum may occur, but its likelihood is remarkably low [46–49]. In a study by Yoshida *et al.* of 80 human breast milk samples, HPV16 was detected in 2.5% of samples [46], while in a study by Sarkola *et al.* of 223 human breast milk samples collected at 3 days postpartum, the rate of HPV16 detection was 4.0% [47]. In a study by Mammas *et al.* of human breast milk samples collected from 21 HPV-positive and 11 HPV-negative mothers, no high-risk HPV16,

18, 31, 33, 35, 39, 45, 51, 52, 56 or 58 DNA was detected [48]. HPVs in human breast milk have not been found to be related to the presence of HPVs in the oral cavity of children [46]. This major observation indicates that HPVs are not vertically transmitted by breast-feeding. However, the presence of HPVs in human breast fluids suggests their potential role in breast carcinogenesis.

HPVs in nongenital cancers: two principal paradigms

HPVs & breast cancer oncogenesis

Breast cancer is the most common female cancer and the third most common cause of cancer deaths worldwide [50]. It is a multifactorial disease, possessing various risk factors, which include hormonal, genetic and environmental factors [51]. A number of studies have suggested a possible relationship between breast cancinogenesis and viral infection, particularly with mouse mammary tumor virus, simian virus 40, EBV and HPV [50,52]. To date, several researchers have presented increasing evidence for the presence of HPVs in human breast cancer specimens [50]. These observations have suggested a possible role for HPVs in the pathogenesis of breast cancer, indicating a causative role for high-risk HPVs in human breast cancer and offering the possibility of primary prevention of some breast cancers by vaccination against HPVs [53].

In a meta-analysis performed by Li *et al.*, 24.49% of breast cancer cases were associated with high-risk HPVs, with a rate of 32.42% occurring in Asia and 12.91% in Europe [54]. The most commonly identified HPV types were HPV33, 18, 16 and 35. In addition, the analysis of ten case–control studies containing 447 breast cancer cases and 275 controls showed a significant increase in breast carcinoma risk with HPV positivity [54]. In a systematic review by Simões *et al.* of 29 primary studies, including 2211 samples, the prevalence of HPVs in patients with breast cancer was 23.0%, ranging from 13.4% in Europe to 42.9% in North America and Australia [55]. Recently, Antonsson *et al.* reported a 50% proportion of HPV-positive breast cancers detected in their series using fresh frozen tissues, with sequence analysis indicating all cases to be positive for HPV18 [56].

The question that remains is whether HPVs are a causative trigger or just a coincidence [57]. Studies of HPV-related koilocytes in breast cancer have provided evidence towards addressing this crucial issue [57,58]. Koilocytes are commonly present in cervical intraepithelial neoplasia and are accepted as pathognomonic of HPV infection in the human cervix. The presence of putative koilocytes in the breast skin and cancer tissue of patients with ductal carcinoma *in situ* and invasive ductal carcinomas indicates that HPVs may be causally related to breast cancer [57]. Moreover, it has been shown that the oncogenic characteristics of HPV-associated breast cancer, including koilocytes, present similarities to HPV-associated cervical cancer [58]. These findings are of great importance, since the majority of current studies demonstrating the presence of HPV DNA in human breast cancer specimens have relied on conventional PCR, a method that is susceptible to genomic contamination [58].

However, other researchers have reported no association between the most prevalent types of HPVs and breast cancer [51,59,60]. These conflicting data do not allow the establishment of a definitive relationship between human breast cancer and HPV infection. The increased sensitivity and specificity of modern molecular techniques will contribute to the understanding of the inherent challenges in detecting HPVs in breast cancer tissue. The effectiveness of the current vaccines against high-risk HPVs as an option for breast cancer prevention should be explored in future studies.

HPVs & lung cancer oncogenesis

Lung cancer is considered to be the leading cause of cancer mortality worldwide [61]. Non-small-cell lung cancer (NSCLC) is a heterogeneous disease, including squamous cell carcinoma, adenocarcinoma and large-cell carcinoma [61]. Despite different histological types, NSCLCs are often classified together because of similarities in approach and management of the disease. The association between tobacco smoking and lung cancer has been suggested for more than 50 years and continues to be the dominant cause of this malignant disease. Inherent predispositions to the disease have long been suspected, and recent investigations suggest several potential mechanisms and a possible mode of inheritance [62].

To date, infection with specific high-risk HPV16 and 18 has also been strongly associated with the genesis of lung cancer [63–78]. In a meta-analysis by Srinivasan *et al.* of 37 published studies, including 2435 cases with primary lung cancer, the overall HPV prevalence ranged between 0 and 78.3%, with a large heterogeneity across geographic regions and histological tissue types [63]. A higher proportion of the European studies reported a less than 10% prevalence of

HPV infection compared with the Asian studies [63]. In a review by Klein *et al.* of 53 publications reporting on 4508 cases, the mean incidence of HPVs in lung cancer was 24.5% [64]. The average reported frequencies in Europe and America were 17 and 15%, respectively, while the mean rate of HPVs in Asian lung cancer samples was 35.7% [64]. Particularly high frequencies of up to 80% were seen in certain countries and regions, such as Japan and Taiwan [64]. The presence of HPV in lung tumor specimens has been found to be higher compared with normal or benign lung controls [65,66].

Recently, in an analysis of 176 lung squamous cell carcinomas and 128 lung adenocarcinomas from Asia by Goto *et al.*, HPVs were found in 6.3% of patients with lung squamous cell carcinoma and in 7% of lung adenocarcinomas [67]. In a study by Baba *et al.* of 27 lung squamous cell carcinomas and 30 lung adenocarcinomas from a southern area of Japan, HPVs were found in 7% of squamous cell carcinomas and in 30% of adenocarcinomas [68]. In a study by Aguayo *et al.* of 60 lung carcinomas from the China, Pakistan and Papua New Guinea, HPV16 was detected in 44% of lung squamous cell carcinomas, while the respective rates for adenocarcinomas and small-cell lung cancers were both 0% [69].

HPV oncoproteins E6 and E7, which are critical for cervical carcinogenesis, are frequently expressed in lung carcinomas [70]. HPV16/18 E6 oncoprotein is expressed in lung tumors and is related to p53 inactivation [71]. Recently, it has been proposed that transcriptional activation of hTERT by the E6 oncoprotein is required for HPV16/18-infected lung oncogenesis [71]. Mutations of the *p53* gene and HPV infection may facilitate each other in the generation of lung squamous cell carcinomas [72]. Expression of the HPV16/18 E6 oncoprotein in patients with stage I NSCLC has been related to a higher 5-year cumulative survival rate [73].

Several researchers have examined the effects of HPV oncoproteins E6 and E7 on angiogenesis in NSCLC and their underlying mechanisms [74]. It has been proposed that overexpression of HPV16 E6 and E7 oncoproteins in NSCLC cells significantly promotes angiogenesis both *in vitro* and *in vivo* [69]. HPV infection-induced IL-17 levels can stimulate Mcl-1 expression through the PI3K pathway and promote lung tumor cell progression through a p53- and IL-6-independent pathway [75].

The disease-specific survival has been proposed to be twice as long for individuals with HPV-positive tumors than those with HPV-negative tumors [76]. HPV typing has also been proposed as a very useful diagnostic tool to discriminate primary from metastatic squamous cell carcinomas of the lung [77]. Recently, the possibility of identifying HPV infection in the exhaled breath condensate of lung cancer patients was demonstrated [78].

The 'Trojan horse' oncogenic strategy of HPVs

The Trojan horse was a tale from the Trojan War about the strategy that allowed the Greeks to finally enter the city of Troy and end the conflict. When the Greeks wanted to penetrate the city of Troy, they constructed a large, wooden horse with a hollow interior to house the Greek warriors. They towed the horse to the city walls and presented it as a gift. Thinking they had won the war, the Trojans pulled the horse inside the city and celebrated their victory. The triumph of the Trojans ended in the night, when the Greek warriors vacated the horse, fanned out across Troy and established a stronghold. Similarly, silent infection of high-risk HPVs in childhood may be the key to HPV persistence in human tissues such as the lung and the breast.

Future perspective

Current evidence is strong enough to support the notion that high-risk HPVs can be transmitted from mother to child perinatally. Most of the mucosal high-risk HPV infections in infants are silent infections in their oral cavity. Keeping in mind the presence of high-risk

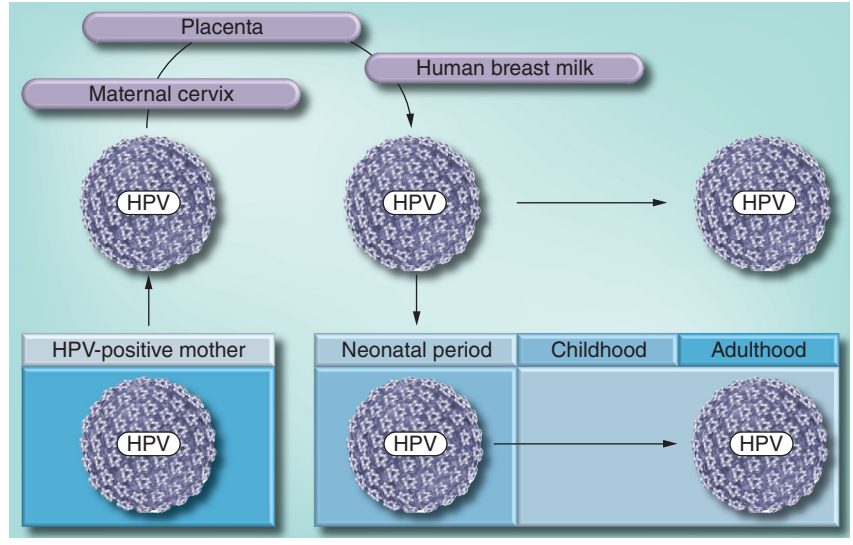

Figure 1. Possible implications of HPV infection for childhood in human oncogenesis.

HPVs in breast and lung tissues, the question of the role of childhood in the transmission of high-risk HPVs is essential. A postulated pathway can be seen in Figure 1. First, high-risk HPVs may infect the female cervix and infection may persist during pregnancy. The second transmission step involves transmission from the high-risk HPV-positive cervix to the neonatal oral mucosa. This transmission can be performed perinatally or via maternal breast milk. The close maternal–newborn concordance indicates that an infected mother may also transmit high-risk HPVs to her newborn via the placenta or cord blood. Early in life, high-risk HPV infection may either clear or remain silent and persistent for a considerable period. The precise pathways that high-risk HPVs use to locate and infect breast and lung tissues are not yet clear; however, it seems that high-risk HPVs in childhood may be responsible for causing the initial step in a series of steps required for cancer development. The point that children seem to be a unique reservoir of silent high-risk HPV infection, analogously to the Trojan horse, should be further investigated.

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Executive summary

HPVs

- HPVs are dsDNA viruses that comprise a remarkably heterogeneous family of more than 130 types targeting the human epithelium.

Detection & clinical impact of HPVs in childhood

- HPVs are common pathogens related to a wide range of cutaneous and mucosal infections in childhood in both boys and girls, such as common warts, genital warts, squamous intraepithelial lessions and recurrent respiratory papillomatosis.

Evidence of HPV perinatal transmission

- In contrast to the consistent epidemiologic evidence of the role of sexual transmission of HPVs in adults, current evidence in infants supports perinatal transmission as the principal mode of HPV transmission in early childhood.

HPVs in nongenital cancers: two principal paradigms

- To date, several researchers have presented increasing evidence for the presence of HPVs in human breast and lung cancer specimens, suggesting the possible role of HPVs in the pathogenesis of nongenital cancers.

The 'Trojan horse' oncogenic strategy of HPVs

- This review suggests that silent HPV infection in childhood, analogously to the Trojan horse, may be the initial step in the oncogenic strategy of high-risk HPVs in humans.

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