Established and potential strategies against papillomavirus infections

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Anogenital cancers, in particular cancer of the cervix, and common, genital and laryngeal warts are primarily caused by infection with human papillomaviruses. Traditionally, the primary goal of treatment is to remove the neoplasia by various surgical approaches; however, all of these have high rates of recurrence. Only a few non-surgical treatments have found their way into clinical practice, and none of them is generally recommended because of side effects, limited efficacy and recurrences. This article summarizes the research on pharmaceutical and immunological approaches that may find a place in clinical practice to complement or replace surgical treatments.

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Introduction

Human papillomaviruses (HPVs) infect mucosal and cutaneous epithelia, and cause malignant and benign neoplastic lesions, such as genital, laryngeal and common warts, and cervical cancer and its precursor lesions. HPVs cause 250,000 deaths per year from cervical cancer, most often in developing countries. This major public health problem, and the even higher incidence of cosmetic lesions, make them important targets in the search for antiviral strategies. Close to 100 HPV types have been formally described by isolating and sequencing the 8 kb circular double-stranded DNA genome. Specific HPV types are normally associated with specific pathological conditions, e.g., HPV-16 and -18 with cervical cancer, HPV-2 and -27 with common warts, and HPV-6 and -11 with genital and laryngeal warts. The molecular biology of HPVs is, in spite of their small genomes, very complex, but can be simplified as follows. Two oncoproteins, E6 and E7, have pleiotropic functions, which include targeting the p53 and RB tumour suppressor proteins. HPVs modulate their own transcription with the virally encoded factor E2 and their replication with E2 and E1. The L1 protein forms capsomers, which arrange into non-enveloped virions. Expression of the HPV genes is controlled by clusters of cis-responsive elements in the long control region, which are regulated by numerous cellular factors.1

Traditional treatments aim at the removal of HPV lesions. Specific strategies are determined by the nature of the lesion and include major invasive surgery, e.g., hysterectomy for metastatic cervical cancer, loop excision or conization of cervical precursor lesions, ablation of external cutaneous lesions by cryotherapy, laser, topically applied corrosive substances such as trichloroacetic acid, and by the activation of cytotoxic substances such as haematoporphyrin derivatives or 5-aminolevulinic acid under the influence of light (photodynamic therapy).2 While these surgical procedures remove the neoplasia, growth usually recurs due to persistence of the virus in healthy tissue.

As a consequence, new antiviral strategies aim to go beyond the removal of the tumour and to have an impact on the amount of latent and subclinical HPV DNA in order to reduce the rate of recurrence. Only a few drug-based anti-HPV strategies are approved for use in clinical practice, but none of these is generally recommended because of side effects and limited efficacy.3 However, numerous potentially powerful approaches are being actively studied. They can be classified into four groups: (i) general stimulation of the cellular immune system; (ii) targeting viral replication, transcription and transformation; (iii) prophylactic vaccination; and (iv) therapeutic vaccination.

Interferon

All interferons (IFNs) have anti-HPV activity, although the typical mediator of the IFN response, double-stranded RNA, is not known to be generated during the HPV life cycle. IFNs have been used for the treatment of laryngeal papillomas, cutaneous and anogenital warts. Partial and total remissions have been achieved with topical, intralesional and systemic administration. While IFN-α is clinically approved for the treatment of genital warts and in some nations is regarded as an essential part of treatment, it is generally not recommended, as the doses required for clinical effects are not tolerable by patients.3

Imiquimod

Imiquimod (Aldara; 3M Pharmaceuticals, St Paul, MN, USA) is a potent adjuvant for the topical treatment of HPV lesions as it generates IFN from plasmacytoid dendritic cells, and interleukin-12 and tumour necrosis factor from myeloid dendritic cells by binding to toll-like receptor 7, thereby biasing to a Th1 cell-mediated immune response.4

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**Indole-3-carbinol**

In clinical studies, as well as in animal models, oestradiol and 16α-hydroxyoestrone were found to support laryngeal as well as cervical neoplasia under the influence of HPV-11 and HPV-16, respectively, although these steroids (in contrast to progesterone) are not known to have a direct effect on HPV biology. In contrast, 2-hydroxyoestrogen is an antiproliferative, and indole-3-carbinol, which induces 2-hydroxylation, antagonizes the proliferative effects of oestradiol and prevents neoplastic growth of the HPV-infected cells.5 Unfortunately, in spite of these encouraging data, clinical Phase II studies of this compound have so far been disappointing.6

**Cidofovir**

Acyclic nucleoside phosphonates, such as cidofovir, were originally identified as antiviral drugs that specifically inhibit certain viral DNA polymerases, such as those encoded by herpes viruses, while they do not influence the cellular enzymes. Subsequently, it was observed that they also have strong activity against HPV lesions, and they are approved for intra-lesion application to laryngeal papillomas. The effect of cidofovir is unexpected, since HPVs do not encode any polymerase. E1 (together with the E2 protein) recognizes and unwinds the viral replication origin, while all subsequent DNA polymerization steps are catalysed by the cellular replication machinery. Further studies indicated that cidofovir not only interferes with HPV biology, but also has an antiproliferative effect against HPV-bearing as well as HPV-free rapidly proliferating tumour cells. These observations point to cidofovir-associated functions beyond the inhibition of viral DNA polymerases, as suggested by the restoration of p53 activities in tumour cells.7,8

5-Fluorouracil

5-Fluorouracil is, like cidofovir, not known to have a specific molecular target in the papillomavirus life cycle, but has been reported to specifically eradicate genital papillomavirus precursor lesions.9 It is not approved for treatment of precursor lesions, although it has been studied as a chemotherapeutic agent against cancer of the cervix, where surgery and/or a combination of radiotherapy and chemotherapy with cisplatin are standard therapies.

**Podophyllin**

Podophyllin resin and its purified derivative podophyllotoxin are antimitotic agents that destroy papillomavirus lesions after topical application by inducing tissue necrosis. In spite of this potency, their use is not recommended, since clinical studies have reported a large variety of adverse effects and recurrence rates of up to 65%.3

**Micronutrients**

Cervical cancer chemoprevention agents under study include diet and micronutrients. There is extensive literature on the effect of retinoids such as β-carotene and vitamin A on the growth of epithelial cells. Recent publications report a substantial number of studies of the effect of retinoids on HPV biology and HPV-immortalized cell lines. Among these are epidemiological studies that suggest a protective effect of this group of substances against progression of HPV lesions. The underlying mechanisms of these effects remain poorly understood, however, and cellular receptors, p53 and virally encoded proteins have been discussed as potential targets.10

There is only inconclusive evidence for a protective role of several other micronutrients, e.g., folates,11 which provide methyl groups, and the antioxidant vitamin C, which also appears to influence methyl metabolism. It will be worthwhile to re-evaluate the clinical findings in view of the recent report that HPV-16 genome methylation represses HPV oncoprotein expression and is much more prevalent in asymptomatic infections than in lesions.12 This latter finding excludes the use of methylation inhibitors in the treatment of cervical cancer. In the case of some other tumours, such a strategy aims at the transcription activation of repressed tumour suppressor genes, while in the case of HPV-induced lesions, it would stimulate HPV oncoprotein expression.

**Strategies for small molecule-based screens and rational drug design**

Cellular transformation by the E6 and E7 proteins and viral replication and gene expression by the E1 and E2 proteins are the most obvious functions that could be targeted by small molecule-based approaches to identify anti-HPV drugs. Unfortunately, there are numerous obstacles to developing these simple concepts into rational drug design or high-throughput screens.

(i) In spite of the notion that E6 and E7 bind two and one zinc ion, respectively, likely giving rise to zinc fingers with the unusual size of 29 amino acids, no structure of either of these two proteins has yet been determined by nuclear magnetic resonance spectroscopy or X-ray crystallography, an obvious obstacle for a rational drug design. It is possible, however, to use the properties of these proteins to bind zinc ions and to interact with certain cellular ligands to design molecularly based high-throughput assays by quantifying the effect of small molecules on the release of zinc ions from E6 and E7, or by quantifying the interruption of intermolecular complexes between E6 and E7 and cellular targets in BIAcore assays.13

(ii) The transcription factor E2 is an inappropriate target as it has activating as well as repressing functions, and it may be impossible to determine which one of these two functions may be altered by E2-interacting chemical compounds. E1, in contrast, may be a useful target, as it is the principal regulator of viral replication, and, with its helicase and ATPase activities, an HPV protein with enzymatic functions that can possibly be exploited in molecular screens.14 HPVs do not encode any other enzymes, notably no proteins with proteolytic or nucleic acid polymerization functions, which could be exploited for screens or by conclusions based on drugs against analogous functions of other viruses.

(iii) As our knowledge of the molecular drug targets of HPVs is of limited use, it has been proposed to base high-throughput screens on the measurement of more general parameters, such as decreases in the high growth rate of HPV-transformed cells or decrease in HPV transcripts under the influence of lead compounds.14

**Macromolecules targeting HPV transcripts**

There is extensive literature indicating that HPV transcripts can be targeted efficiently in vitro by macromolecules such as ribozymes,15 siRNA16 and antisense RNA,17 with the consequence of decreased growth of HPV-dependent cell lines. Unfortunately, these specific approaches share the same dilemma of all research into the development of macromolecule-based and biodegradable therapeutic sub-
stances, namely delivery at specific anatomic sites of therapeutically active concentrations following systemic application.

Prophylactic vaccination

Prophylactic antiviral vaccination has been one of the greatest successes of medicine and has led to the eradication or regional reduction of the incidence of severe and life threatening diseases like smallpox, polio, mumps and measles, and the combat of HPV infections may have a similar solution. A recent clinical Phase II study of vaccination with HPV-16 virus-like particles for induction of an IgG-based anti-L1 response has been a major success, as the vaccine protected all immunized women over an average test period of 17.4 months against HPV-16 infection, while 3.8% of the placebo recipients became infected.\(^1\) The duration of the protection by this vaccination is not yet known, however, and identifying the optimal age for vaccination will be a top research priority. However, the potential need for repeated vaccinations throughout adult life may make even a successful vaccination protocol difficult for the general public to accept.

Therapeutic vaccination

The HPV oncoproteins E6 and E7 are suitable targets for cell-mediated immunity, as they are necessary for establishment and maintenance of the tumorigenic state. A large number of reports describe the use of pure E6 or E7 proteins, E6 and E7 peptides, fusion proteins with hsp70 and other leader proteins including the papillomavirus L1 capsid protein, DNA-based vaccination, prime-boost approaches, and chimeric viruses expressing these HPV oncoproteins, such as vaccinia. In some of these reports, specific cell-mediated immune responses in experimental animals led to suppression of HPV-induced tumours. Several ongoing clinical trials have been designed in the hope that similar vaccinations of humans may suppress HPV lesions by cell-mediated immunity.\(^19\)

Conclusions

The last 20 years of molecular research have led to a thorough understanding of the biology of HPVs. This has led so far only to three compounds, IFN-\(\alpha\), imiquimod and cidofovir, the use of which in clinical practice is approved, but not recommended, because of limited efficacy, high recurrence rates and side effects. None of the therapeutic strategies presently under research is sufficiently advanced to dramatically improve this picture in the next few years, while in the long run (i.e. over a time span exceeding 10 years), combinations of prophylactic and therapeutic vaccination, a better understanding of anti-HPV nutrients, and the detection of small antiviral molecules will provide great opportunities to decrease subclinical infections and expand the pharmaceutical arsenal to control cancer and cosmetic lesions.

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References