Chapter

E1 and E2 Viral Proteins as Therapeutic Targets for Development of Antiviral Agents

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Abstract

The importance of studying the human papillomavirus (HPV) is because it is a disease that relies on 14 HPV types classified as carcinogenic high risk and that contributes to cervical cancers affecting approximately 527,600 women yearly and causing 265,387 deaths yearly, being the second mortality cause for women globally. In Mexico, 13.9% of demises are due to cervical uterine cancer (CUCA). The challenges for a vaccine that may prevent HPV occurrence are an active field for scientists with significant advances but still undergoing for a full cure to this disease. In this work, latest research trends to treat HPV are analyzed, and by means of molecular coupling analysis, a modeling and simulation process to predict interactions of leader molecules with the target for synthetic elaboration of a possible therapeutic treatment is developed. One of the main topics discussed in this chapter relates to new drug design for HPV treatment, which is related to the inhibitors of protein-protein interactions and in the protein drugs. Regarding HPV therapy development, a group of small molecules has been identified using high-performance sieving capable of interrupting HPV16 E1-E2 interaction, which helps avoid viral replication. Some of these compounds displayed nanomolar affinities and high specificity.

Keywords: viral proteins, human papillomavirus, HPV, E1-E2 proteins, TDDFT

1. Introduction

Human papillomavirus (HPV) belongs to the Papillomaviridae family, according to the eighth report developed by the International Committee for Viral Taxonomy. To date, over 100 different HPV types have been cataloged and around 30 infected anogenital mucosae. HPV types are classified as virus from low to high risk according to their capability to cause benign or carcinogenic injuries, respectively [1].

Infection provoked by HPV is one of the more common causes among sexually transmitted diseases with prevalence levels varying between the population and geographic location.

At least 14 types of them are classified as carcinogenic high risk (hr-HPV); among them HPV16 and HPV18 are responsible for around 70% of all cervical cancers affecting approximately 527,600 women yearly and causing 265,387 deaths yearly, being the second mortality cause for women globally [2]. Over 85% of new cases and deaths referred occur in developing countries [3] such as Mexico, where 13.9% of demises are due to cervical uterine cancer (CUCA) [4].

The challenges for a vaccine that may prevent HPV occurrence is an active field for scientists with significant advances but still undergoing for a full solution that can eliminate this disease.

In this work latest research trends to treat HPV are analyzed, and by means of molecular coupling analysis, a modeling and simulation process to predict interactions of leader molecules with the target for synthetic elaboration of a possible therapeutic treatment is developed.

This chapter is a review of the latest advances on new drug design for HPV treatment which are related to the inhibitors of protein-protein interactions and in the protein drugs. Regarding HPV therapy development, a group of small molecules has been identified using high -performance sieving capable of interrupting HPV16 E1-E2 interaction which helps avoid viral replication. Some of these compounds displayed nanomolar affinities and high specificity.

2. HPV genome, treatment, and prevention

2.1 HPV infection, treatment, and prevention

Currently, an antiviral treatment is unavailable that can help eliminate the infection or avoid progression of injuries caused by CUCA. Current therapies for cervical dysplasia and cancer implicate destruction or elimination of infected tissue by cytotoxic agents or surgery [5]. On the other hand, FDA has approved three vaccines for HPV infection prevention: Gardasil, Gardasil 9, and Cervarix. All three prevent HPV infection types 16 and 18 [6, 7]. Gardasil also protects HPV infection types 6 and 11, which cause 90% of genital warts [8]. Gardasil 9 protects from infection of the same four HPV types and other five HPV types (31/33/45/52/58) that cause cancer [9]. These vaccines are based in empty particles similar to the "ghost" virus, formed by L1 protein capsid from each viral type [8, 10]. It has been demonstrated that these vaccines are highly protective against the lesions of transformed cells at an early stage that represent cervical cancer precursors. Since HPV 16 and HPV 18 currently represent the two HR-HPV more common in most geographical locations, universal adoption of the vaccines may protect more than two-thirds of HPV-induced cancers [10].

However, vaccination programs are not implemented worldwide, and coverage is still very low especially in low-income countries [10, 11].

It has been estimated that the addition of HPV 31/33/45/52/58 to the nine-valent vaccine could prevent almost 90% of cases of invasive cervical cancer worldwide [12, 13].

HPV vaccines currently available are useful tools in the fight against HPV infections and cancers but provide an incomplete answer for long-term HPV disease eradication. By their very nature of being capable of inducing a strong and protective humural—but no cellular—host immune response, they are also of little therapeutic value to already infected patients [10].

Scientists are working in new HPV therapeutic vaccines which may help avoid cancer formation in women previously infected with HPV [14]. These vaccines work by stimulating the immune system to attack and destroy infected cells. Clinical studies under development are evaluating safety and efficiency of a DNA

therapeutic vaccine to treat injuries by HPV in the cervix and vulva. An ideal strategy would be to combine a preventive and a therapeutic vaccine. Another prevention strategy under testing is using external microbicidal. It has been found that carrageenan, a compound that can be extracted from seaweeds and extensively used in foods and other products, inhibits HPV infection in laboratory studies. A clinical study is under development to demonstrate if a gel containing carrageenan is capable to prevent genital infection by HPV in healthy individuals [15–17].

2.2 HPV genome

HPV genome (**Figure 1**) consists of a circular double-chain DNA molecule of approximately 8 Kb in size. The genome is organized in three regions. The region early contains the genes E1–E8 that code predominately for regulatory proteins essential for viral transcription and replication, as well as cell cycle control; the late region encodes for the two viral structural proteins, L1 and L2, needed for capsid formation and the noncoding region also known are long control region (LCR) or upstream regulatory region (URR). The LCR region varies in size between 800 and 1000 pb for the different HPV types, does no encodes any protein but, contains Cis-elements required for regulation of genes expression and genome replication [18].

2.3 HPV life cycle

HPV life cycle is coupled to the differentiation cell program experienced by epithelium keratinocytes. PVs are highly specific from the species they infect and present a tropism defined by epithelial scaly cells infecting undifferentiated basal cells.

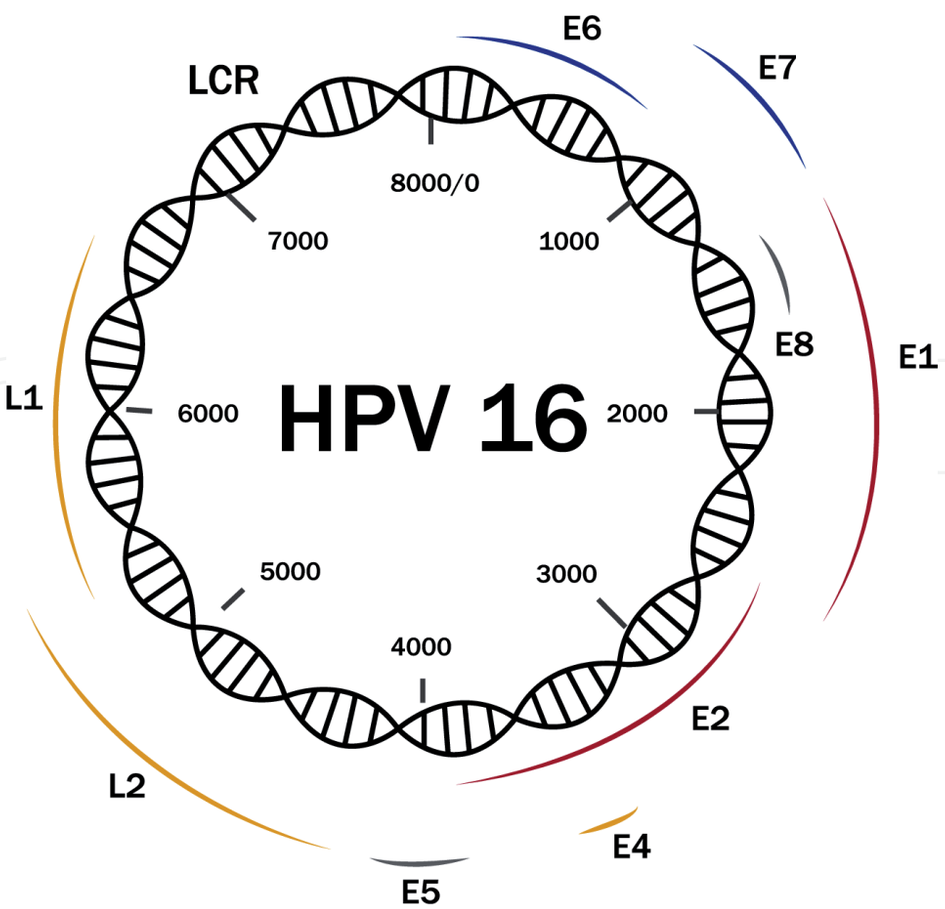

Figure 1.
Representation of HPV genome organization in a circular chain displaying the DNA molecule. LCR is the region noncoding the open reading frames (ORFs) encode the early (E) and late (L) viral proteins [18].

During epithelium mucosa infection, firs of all, virions need to reach the undifferentiated basal cells. Once in contact with blank cells, virion is associated with putative receptors such as alpha integrins, heparins, and laminins probably through wounds or microabrasions that give access to basal zones at the epithelium [19].

Heparin sulfate proteoglycans (HSPGs) from the cellular surface seem to be the primary receptor for initial binding [20]. The joint together is mediated by the last 15 amino acids from the extreme carboxyl-terminal of L1 protein [21]. Virions enter the cell by endocytosis by using the clathrin-dependent route. Decapsidation of the particle occurs at the endosome, releasing the capsid genome and proteins in the endocytic vesicles [22].

After HPV infects basal cells, viral genome is established as a multiple copies episome at the cells' nuclei through several viral DNA replication cycles.

Conservation of the viral episome at 50–100 copies at these undifferentiated cells is obtained then through low DNA replication levels, and it is essential for the infection persistence; by itself it is a risk factor for carcinogenesis induced by the virus; the first proteins to express themselves are the viral replication proteins E1 and E2. E2 protein presents a characteristic regulating function affecting the transcription, the replication, and the episome conservation [20, 23].

During the productive infection, viral protein detection at the basal layers barely exists, even though E7 protein may be detected by cervical neoplasia, where the expression levels cannot be controlled properly [23]. Proliferative basal cells would migrate to parabasal and intermediate layers, starting the differentiation program and with it the transcription of the different early viral genes, regulated through the LCR region. During this period DNA will replicate hundreds of copies by cell, thanks to E6 and E7 proteins which will block the exit of cells from the cellular cycle [24].

During productive infection, HPV proteins cannot easily be detected in the basal cell layer, although the E7 protein can be seen in cervical neoplasia where expression levels are not properly controlled.

As HPV-infected cells divide and differentiate, the late HPV promoter is activated mediated by transcription factors dependent on the differentiation, this has as consequence the amplification of the viral DNA, and an increase in the levels of viral proteins needed for replication, including E1, E4 and E5, being E4 the more abundant.

It has been suggested that E7 continuous expression in a cell containing abundant E4 which favors the conservation of the cell in phase S, this allows the viral genome accumulation.

When basal cells enter the differentiation process that will convert them into keratinocytes, at the same time they migrate to superior layers in the epithelium that will cause an explosion in the viral DNA replication, known as vegetative replication.

Finally, both proteins and the capsid (L1 and L2) are expressed only in cells that have passed through the vegetative viral amplification. At the end of the productive cycle, genomes will encapsidate, generating virions that will be let out with release of superior layer cells from the mucous epithelium [23].

HPV DNA replication initiates with the cooperative join of E1 and E2 with specific DNA sequences inside the viral origin [15–17]. Formation of this ternary compound E1-E2-ori depends on the interaction between both proteins with DNA, but also there is a critical interaction between the E2 transactivation domain of N-terminal (TAD) and the E1 enzymatic domain of E1 C-terminal [25]. Assembly of this initial complex E1-E2-ori is the starting point for additional E1 molecule recruitment [26, 27] and its assembly in hexamers and double hexamers that display activity in ATPase and helicase [28, 29]. Any of the protein–protein and

protein-DNA interactions produced in the origin could be directed toward the development of small antiviral molecules to treat infections by HPV.

It has been demonstrated clearly that expression E1 and E2 proteins from different HPV genomes can induce replication of the genomic integrated HPV origin [30], and this is a particular danger in the presence of DNA damage [31]. Then, anti-HPV agents unblocking viral DNA synthesis could, in time, benefit cancer development increasing HPV genome integration. Due to E1 and E2 capability to induce HPV DNA integration, HPV DNA replication through E1 interactions with cellular replication proteins probably may represent one of the best approaches to develop an HPV antiviral that also prevents HPV integration at the host cell chromosomes.

2.4 E1 and E2 proteins as therapeutic targets

E1 is the most conserved protein among the papillomavirus and the only with enzymatic activity [32, 33]. This situation adds up to the fact that viral DNA replication is absolutely dependent on E1, and it has contributed to do an attractive target out of this protein so antiviral agents may be developed.

E1 gene is the bigger and one of the more conserved PVs with approximately 2 Kb, and it codifies E1 multimeric protein [28].

E1 protein has three functional domains. C-terminal domain has helicase/ATPase, and it participates in viral DNA replication and includes sequences involved in the protein oligomerization [34, 35].

Variable length central region contains the DNA-binding domain (DBD), which recognizes specific sites at the ori but with low affinity [36–38], and an N-terminal regulator region which is essential for live optimal replication [39, 40].

The last one codifies several functional elements, such as a nuclear localization sequence (NLS), a nuclear export sequence (NES), a joint cyclin motif, and Cdk2 phosphorylation sites. E1 N-terminal regulator region for HPV anogenital type also interact with cellular p80 protein known alternatively as UAF1 or WDR48 [41].

Terminal carboxyl domain and spacer region have been widely studied. However, N-terminal domain functions have not been well defined other than those related to the nuclear location [35].

DBD of E1 protein consists in a sequence of 60 pb approximately. This sequence contains three elements, a sequence rich in A and T, a palindrome sequence found at LCR, forming hexamers and double hexamer, and a 12 pb sequence that constitutes a joining site for E2 protein [42, 43].

DBD has been widely characterized in vitro and it was the first E1 domain to be crystallized [43–45]. And this reveals an unusual joint mode to DNA by an extended bond and an α helix forming a continuous surface as DNA join.

Even though DBD more important function is to recognize and mark the ori, it has other functions as well. DBD performs an important role in the fusion of bicatenary DNA and the formation of hexameric replicative helicase [43].

The E1 N-terminal region is formed by 200 amino acids approximately; it is the less preserved segment of this protein. This region contains a number of motifs from short sequence amino acids preserved as variable among different PVs, including a nuclear localization signal (NLS), a nuclear export signal of dependent-Crm1 (NES), and a joining motif to the cyclin (CBM) that interacts with cyclin A/E in complex with kinase dependent of cyclin 2 (Cdk2) and Cdk2 phosphorylation sites and other kinases [40, 46, 47].

The E2 gene has approximately 1100 bp codifies a nuclear protein of 45 kDa [18, 48], E2 protein is composed of three functional domains. The first, at

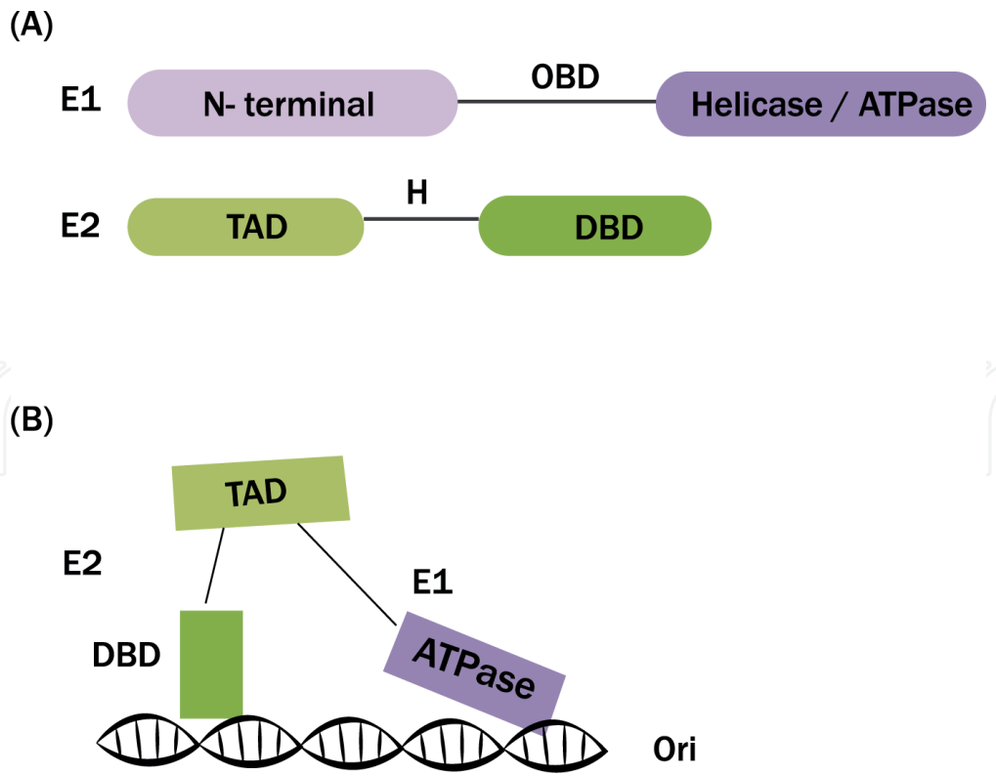

Figure 2.
Initiation of HPV DNA replication. (A) Schematic representation of the viral proteins E1 and E2 required for replication of the HPV genome. E1 and E2 are approximately 650 and 370 amino acids in length, respectively. Locations of the different functional domains in each protein are indicated. OBD, origin binding domain helicase/ATPase N-terminal; TAD, transactivation domain; H, hinge region; DBD, DNA-binding domain. (B) Schematic diagram of the initiation of HPV DNA replication. (I) Replication is initiated by the recruitment of E1 (purple) and by E2 (green), to the viral origin [10].

the extreme amino terminal, is the activation domain (E2TAD), responsible of regulating the transcription and replication of the viral genome [18, 48]. The second domain is a hinge or central domain, with more variable length and sequence among the HPVs. The third domain at the carboxyl-terminal is for dimerization and binding with the DNA, and it is formed by 100 amino acids approximately [18, 48, 49]. Crystal structure E2 protein from all viral strains have in common the fact of joining a palindromic DNA sequence (ACCgNNNNcGGT, lower case letters indicate preferred nucleotides; NNNN region is called spacing region), referred as the E2 union site. Nevertheless, there are specific differences in viral strains in regard to E2 protein ability to discriminate union sites [48] (**Figure 2**).

HPV protein E2 displays independent complex functions regarding the transcription and may be able to modulate host cells with respect to the viral replication cycle [48–50].

3. Computational modeling of E1 and E2 inhibitors

Several pharmaceutical companies have invested resources in the identification of E1 ATPase/helicase inhibitors. Even though small molecules have been identified as capable to inhibit this enzyme activity, unfortunately, none of these projects has resulted in a viable therapy. In late years, antiviral research has begun to deal with the vital interactions modulation between proteins and proteins as a feasible therapeutic way.

An interaction protein–protein essential in the HPV DNA replication is the interaction between HPV E1 and E2 proteins. There are diverse reported works with advances in identification of leader compounds that may interrupt protein–protein E1-E2 interactions. Some of these compounds showed nanomolar affinities and high specificity.

Yoakim and coworker [51] discovered a group of small molecules known as indanones, and this molecules showed the capability to inhibit specifically E1-E2 protein interaction, which also have been identified as the first inhibitors for small molecules capable to antagonize the assembly of E1-E2 complex and the viral ori HPV11. It was identified a compound that inhibited E1-E2 protein interaction by means of high-performance sieving from the collection of over Boehringer Ingelheim 100,000 compounds, using a scintillation proximity assay (SPA). Also, more active analogues were synthesized that were capable to inhibit the E1-E2 complex assembly with the HPV11 viral ori with low nanomolar power in vitro and with activity in cell culture. Structurally, this group of inhibitors presents an indandione system spirofused onto a substituted tetrahydrofuran ring which were denominated as indandione inhibitors. It was determined that these inhibitors act by joining to E2 TAD domain, the same protein region that interacts with E1 [51, 52]. Crystalline structures, HPV11 E2 TAD by itself or as a complex with an indandione inhibitor, both provide a detailed comprehension of the mechanism in which indandione inhibitors join E2 to interrupt the interaction E1-E2. A comparison between both structures revealed that the union between the inhibitors caused insignificant alterations on the protein backbone, but it did induce a significant movement of several lateral amino acid chains at the union site. These changes in the lateral chain conformation, particularly from Tyr-19, His-32, Leu-94 y, and Glu-100 residues, resulted in the formation of a deep hydrophobic bag that is joined firmly to the inhibitor indandione fraction. The structure also revealed that the inhibitor carboxylate fraction, known by its importance for power, forms hydrogen bonds with amides from the protein backbone. Studies regarding the structure-activity relationship (SAR) revealed that the carboxylate fraction is important in potency of the inhibitor. Mechanistic studies, including the use of isothermal titling calorimetry, showed that the indandione inhibitors join reversibly to E2 TAD with 1:1 stoichiometry. Even though indandione inhibitors displayed a powerful activity against E2 proteins of low-risk viruses HPV6 and HPV11, these did not show any activity against high-risk types HPV16, HPV18, and HPV31. Cellular culture studies showed that indandione inhibitors are capable of antagonizing E1-E2 interaction of HPV6 and HPV11 [49].

3.1 Other labs' approach

White and coworker addressed these issues in a series of detailed studies to identify inhibitors of the cooperative assembly of HPV E1 and E2 on the ori, focusing on HPV 6 and 11. They identified two series of inhibitors that bound to overlapping sites at the E1 binding interface on the E2 TAD. Results obtained from modeling works suggest that inhibitors derived from repaglinide form weak interactions with protein E2 TAD but occupy a bigger portion from the inhibitor union bag than the indandione series.

Both series of compounds were optimized for binding by medicinal chemistry approaches, and in both series the best compounds in each series had low nanomolar activity against the HPV 11 E1-E2 interaction.

The fact that the potent repaglinide derivatives, with values IC_{50} at nanomolar range against HPV6 and HPV11 E2, and EC_{50} values in cellular DNA replication

assays of approximately 1 mM, make this series a promising route for development of small molecular inhibitors for E1-E2 interaction [53].

Capabilities of indandione compounds to inhibit HPV genome in vivo replication, especially low-risk HPV types, demonstrate for the first time the therapeutic potential of protein E2 as target for the development of small molecule inhibitors at HPV protein interaction, particularly in the case for treatment of anogenital warts caused by HPV6 and HPV11. Furthermore, location and characterization of the union bag for E2 TAD inhibitors provide a new potential therapeutic option for treatment of HPV infections. These studies have set the basis for the use of approaches based in structures for rational design or for virtual selection of inhibitor compounds capable of joining to all or part of the identified inhibitor union bag.

Kantang and coworker worked in the prediction of the interactions of the helicase domain of the E1 protein of HPV16 and the TAD domain of HPV16; for this purpose, E1 protein domain was modeled (residue 421–622) from HPV16 with I-TASSER server. The model with the best C-score was selected to coupling to structure E2 from HPV16 and HPV18 using ClusPro. Superposition of E1 HPV16 and the crystalline structure of E1 HPV18 showed an RMSD of 1.39 Å, indicating structural similitude. A complex structure for HPV16 E1-E2 is also predicted as similar to crystalline structure of HPV18 E1-E2, with an RMSD value of 1.11 Å. An analysis of HPV16 E1-E2 interactions revealed there are three sites for interaction of complex HPV16 E1 and E2. The residues Glu118, and Tyr178 at the structural domain of folded β-sheet for HPV16 E2 form hydrogen bonds with Tyr578, Arg575, Ser574, Asp573 of HPV16 E1, respectively.

HPV16 E2 helicoidal N-terminal domain is the main union site for HPV16 E1 protein where Asp13, Thr17, Tyr19, Asp22, Tyr32, Glu39, and Val58 from HPV16 E2 interact with Arg615 (for Asp13 and Thr17 E2), Arg447, Arg619, Glu452, Arg447, and Tyr602 from HPV16 E1, respectively. Shows Gln95 and Glu100; at the linking segment between N-terminal and C-terminal HPV16 E2, there are interactions with Arg462 and Ser455 from HPV16 E1, respectively. Besides, structures of nine peptides reported [54] were built by I-TASSER server. Using ClusPro web server, it was able to predict binding, conformations, and interactions of peptides with HPV16 E2 protein; bind conformations and interactions between small peptides and HPV16 E2 protein were predicted and analyzed. These results were used ahead for the design of more powerful peptides that may potentially inhibit E1-E2 complex formation. Binding affinities obtained for designed peptides and protein E2 of HPV16 recombinant were in good agreement with experimental results. Four peptides were the more efficient inhibitors of E1-E2 complex, and they could be used for suppression of HPV replication [55].

4. Conclusions

Currently a cure or treatment for HPV is unavailable. In many men and women, HPV disappears by itself without causing further problems. There are treatments for affections caused by the virus. Among these affections are genital warts, precarcinogenic cells, and cancer.

Even though there are vaccines against the main HPV types, there are therapeutic treatments needed for those that have been already exposed to the virus which represent most of the population affected by this disease. In this regard, a diversity of research work related to antiviral design exists, including those work using computational methods. These studies have laid the foundation for the use of structure-based approaches to rationally design or virtually screen inhibitory compounds that are capable of preventing HPV replication.

By means of molecular coupling analysis, it is possible to predict interactions of leader molecules with the target with enough detail to be useful for synthetic elaboration of those molecules.

It has been considered that protein-protein interactions are difficult to inhibit because they often involve big surfaces, depriving small molecules to bind with the pocket; however, they are receiving great attention to work as targets for the rational design of drugs. The interest in the protein-protein interaction inhibitors and in the protein drugs has been in constant growth.

Regarding HPV therapy development, a group of small molecules has been identified using high-performance sieving capable of interrupting HPV16 E1-E2 interaction which helps avoid viral replication.

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Conflict of interest

The authors state that this research was completed without any conflicts of interest related with the funding to develop the present work.

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