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Design of a Peptide Against the Interaction Between Immune Response Protein TRAF5 and the Oncoprotein E6 from HPV

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Abstract

HPV is the most common sexual transmitted disease worldwide. There is no specific treatment for the disease and the impact of HPV on public health and the necessity for more prominent diagnosis and treatment are reflected by the genital infection and cervical cancer statistics. The disease has a high rate of deaths due to its relation to cancer, principally cervix cancer. Cancerous warts also affect the vulva, vagina, anus, penis, mouth and throat. There are more than 80 HPV types and more than 40 infect the genital tract. Several HPV genes and proteins are potential candidates as genetic markers for the development of cancer and they are target for vaccines and HPV treatment. E6 exerts pleiotropic functions, including signaling, cell cycle regulation, cell line transformation, immortalization of primary cell line and genome stability regulation. The E6 protein interacts with several host-proteins, including TRAF5, which is related to cytokines signaling. E6 induces the destruction of host regulatory proteins through the proteasome system, being an important target for the development of new therapies against HPV. Here, we hypothesize that interaction between TRAF5 and E6 could be modulated in order to inhibit the activity of E6. We have shown an *in silico* approach of interaction between TRAF5 and HPV E6 through the identification of hot spots within the interface of interaction of the complex. We propose a new peptide that interacts and inhibits HPV E6. For a future perspective, the peptide designed will be tested *in vitro* and other regions of the interface of interaction will also be screened so that other peptides could also be designed and tested.

Keywords: HPV; TRAF5; Hot spots screening; Design of peptide

Introduction

According to the World Health Organization (WHO), the human papillomavirus (HPV) affects more than 600 million people worldwide, being the most common sexually transmitted disease (STD). There were over 250,000 deaths due to cervical cancer worldwide and most of them took place in developing countries (WHO). There are more than 80 HPV types and more than 40 infect the genital tract [1]. Chronic infection

with certain HPV subtypes increases cervical cancer susceptibility [2], even though most HPV infections are asymptomatic and warts might spontaneously resolve [3]. The immune system gets rid of HPV within 12-24 months in 90% of the patients [4], either completely or reduced to undetectable levels [5]. Cancerous warts affect mainly the uterine canal and less frequently the vulva, vagina, anus, penis, mouth and throat [6,7]. There is no specific treatment for the disease and the impact of HPV on public health and the necessity for more prominent diagnosis and treatment are reflected by the genital infection and cervical cancer statistics.

HPV infects epithelial cells and it takes around 24 hours for the beginning of viral components transcription to occur, with up to 200 viral genomes in each host cell infected [8]. The immune system cells are activated but viral particles still remain on the cell surfaces, bound to receptors such as annexin, integrin and laminins [9]. The HPV genome comprises 8 Kbp (kilo base pairs) in a circular double DNA strand [10,11]. The viral genome contains two main regions. The late region (L), which codes for the capsid proteins and the early region (E) with sequences that code for proteins related to transcription and cellular transformation. In addition, genes within the E region has a central role as oncogenes and are usually selected as possible target for diagnosis and drug development [12].

It has been shown that several types of cytokines inhibit intracellular HPV proliferation and the expression of the E region genes [13]. The cytokine tumor necrosis factor (TNF) is produced by keratinocytes, which are host to HPV. These cells show anti-proliferative properties on infected cells [14,15]. TNF, transforming growth factor (TGF) and interleukin 1α repress certain types of HPV E6 and E7 oncogenes expression in epithelial cells [16]. Malignant transformation arises with the loss of the inhibitory roles of cytokines due to polymorphisms [17], family history [18], environmental [19] and xenobiotic factors [20]. Keratinocytes express receptors with innate immune system roles on their plasma membrane such as pathogen recognition receptors, thus the immune system recognize pathogen associated molecular patterns [21]. Innate and adaptive immune cells respond to viruses trying to stop the infection and to eliminate the infected cells. However, the pathogens down regulate cytokine responses in order to control cellular metabolism and establish infection.

Some of the proteins coded by the E region of the HPV genome, especially the protein E6, show cell transforming properties [22] and being requested for malignant transformation. E6 exerts pleiotropic functions, including signaling [23], cell cycle regulation [24], cell line transformation [22], immortalization of primary cell line [25] and genome stability regulation [26]. The E6 protein interacts with several host-proteins and induces the destruction of host regulatory proteins through the proteasome system [27]. The protein has 158 amino acid residues and its conformational tridimensional structure is formed by two zinc-finger binding motif, PDZ-binding domain, an endonuclease and a helicase domains [28]. The E6 oncogene might compromise the immune system through down regulation of antigen-presenting cells, induce viral persistence and increased susceptibility of cancer. Moreover, super expression of E6 and other oncogenes increases genomic instability, bringing opportunity to cancer cells scape immune surveillance.

Cytokines regulates the immune response of the HPV infection. An important family of cytokines related to HPV and the viral immune evasion is the tumor necrosis factor receptor-associated factor (TRAF), as it mediates TNF activation. It has been shown that HPV E proteins increases the effect of TNF through TRAF5 activation by direct interaction [29]. In addition, TRAF5 is down regulated in cytoplasmic extracts from cells infected with HPV compared to uninfected control cells [30]. Hence, defects of genes coding for cytokines and other immune system-related proteins produce a favorable microenvironment to HPV replication, lesion endurance and immune evasion, increasing HPV patients' susceptibility to cancer.

Here, we hypothesize that interaction between TRAF5 and E6 could be modulated in order to inhibit the activity of E6. We have shown an *in silico* approach of interaction between TRAF5 and HPV E6 through the identification of hot spots within the interface of interaction of the complex. We propose a new peptide that interacts and inhibits HPV E6 functions.

Materials and Methods

The 3-D structures of the Homo sapiens protein TRAF5 and the HPV E6 used in the analysis are available in the PDB (protein databank; https://www.rcsb.org/). We used KBDOCK and InterPro in order to find the E6 and TRAF5 protein domains [28,31,32]. The TRAF5 interactome was performed on BioGrid and included protein-protein interaction with evidence from genetic and physical experiments. For each interaction in the interactome was set a minimum evidence score of 5, which means that the interaction was validated by at least five different types of approaches [33]. Docking analysis was performed through ClusPro [34]. The procedure is based on Piper [35], which relies on a multi-staged approach and numerical methods to

predict protein-protein interactions. The models of interactions are grouped into clusters according to their size and the they are ranked. The final best model relies on the one that showed lower free binding energy.

We used PyMol (https://pymol.org) in order to analyze the docking results by the visualization of the interface of interaction, predicted hot spots and polymorphic residues. The hot spots in the proteins under study were identified by KFC2 [36]. The analyses are based on the structural microenvironment around amino acid residues and hot spots determined experimentally are taken into consideration. The hot spot prediction takes into account conformation specificity (K-FADE score) and biochemical features such as hydrophobicity, polar and electrostatic interactions (K-CON score) through a machine learning approach. This approach identifies amino acid residues that significantly contribute to the binding free energy within the protein-protein interaction interface. Clinically important polymorphic residues for the TRAF5 protein were identified through the dbSNP (database of single nucleotide polymorphism; https://www.ncbi.nlm.nih.gov/SNP).

Results and Discussion

H. sapiens TRAF5 structure organization

TRAF5 is a protein that takes parts in several biological process, including activation of immune system cells [37]. At least three main factors guarantee the functions performed by the protein: the stability of its three-dimensional conformation, the presence of conserved domains and the protein partners that TRAF5 interacts with (Figure 1). TRAF5 contains three zinc-finger motifs, which form finger-like projections

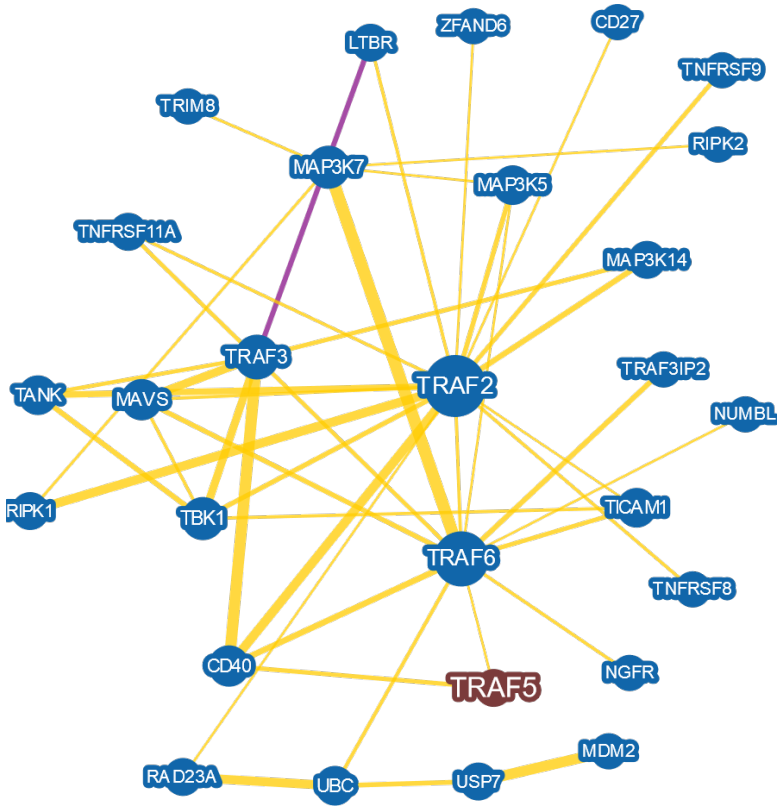

Figure 1: ***Homo sapiens* TRAF5 interactome.** The protein TRAF5 participates in signaling pathways and for this reason it interacts with several proteins within cells. TRAF5 is related to immune response against pathogens, including HPV and may interact with the pathogen proteins. Genetic variation within the nucleotide sequence that codes for the TRAF5 protein is related to disease susceptibility due to PPI disruption and reduction or loss of the protein function. Each blue node represent a TRAF5 protein partner. The yellow edges represent the interaction between TRAF5 and its partners, including neighboring interactions. The thickness of the edges represent the confidence score of the interaction and the purple edge represent an interaction found through genetic evidence. The interactome presented here was retrieved through BioGrid [33].

in order to make interaction with target molecules [38]. A zinc-finger motif often has plastic forms of binding, with active roles on gene transcription and translation [39], mRNA transport [40], cytoskeleton arrangement [41], epithelial development [42], cell adhesion [43], protein folding and interaction with partner [44] and chromatin remodeling [45]. The zinc-finger domain can also regulate several aspects of the immune system roles against pathogens. Certain proteins with this motif act targeting mRNAs and proteins for degradation, consequently controlling signaling pathways in immune response via cytokine synthesis and activation of immune cells [46]. Thus, proteins containing this domain play roles in regulating ubiquitination processes [47,48]. The three zinc-fingers found within TRAF5 structure are located at amino acid residues 45-85, 127-181 and 182-239.

TRAF5 also contain a MATH or TRAF domain in the C-terminus of the protein [49], from the 403 amino acid residue to the 549. This domain participates in self-assembly and interaction performed with other protein partners [50]. The domain forms an antiparallel beta sandwich structure. TRAF5 presents a coiled-coil motif (residues 237-342) near to the MATH domain; this conformation permits proper interaction with targets to form complexes that signals for the TNF receptor-1 during immune response events [51]. Thus, TRAF5 mediates activation of NF-kappa-B (nuclear factor kappa B) and JNK (Jun N-terminal kinases) through these domains.

Approximately 22% of the TRAF5 structure has helical shape and around 31% is beta-sheet (Figure 2). Figure 2A shows the surface structure of TRAF 5 and figure 2B shows details on the secondary structure with helices, alfa sheets and loops. Figure 2A and 2B also shows the region where interaction with E6 from HPV is most stable. Important residues within this region accounts for the low free energy of binding between these host and pathogen proteins. The docking results showed that best cluster, consequently with the lowest energy, had a score of -791.7 (data not shown). The results and scientific evidences support our model predicted for the conformational structure of TRAF5 complexes with HPV E6.

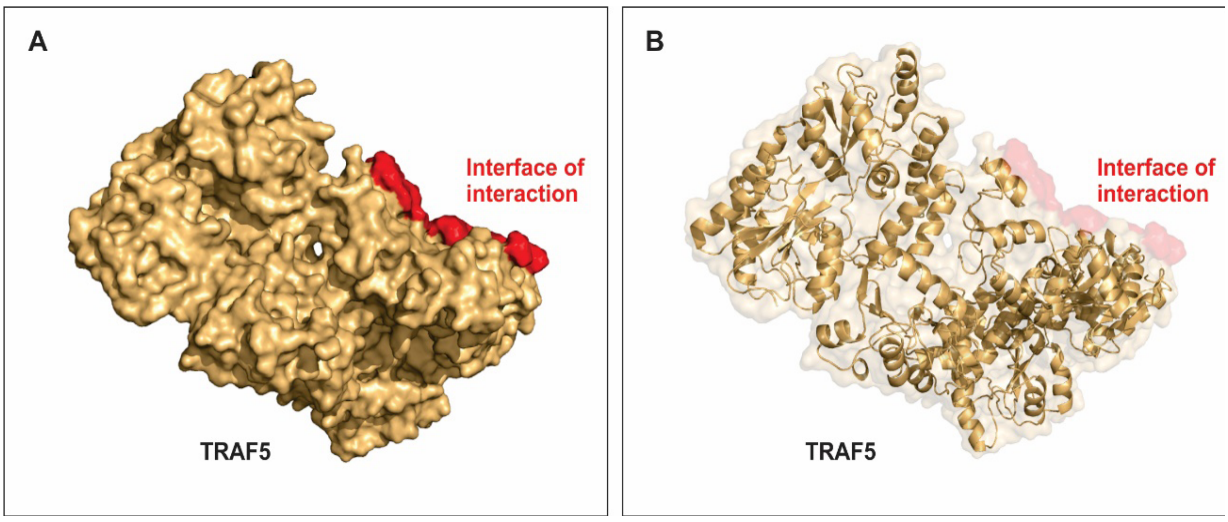

Figure 2: **Three-dimensional conformational structure of TRAF5 when bound to HPV E6.**

A – Representation of the TRAF5 surface structure. Amino acid residues marked by the color red highlight the interface of interaction between TRAF5 and HPV E6, which is considerably large. B – Representation of the secondary structure of TRAF5, which contains alfa-helices, beta-sheets, loops and domains that account for most of the structure and guarantee protein function and multi-protein complex formation.

HPV E6 structure organization

HPV infection induces host cells immortalization, which contributes immune response evasion and cancer development. Two main HPV genes stay hidden within host genome, making cells expresses the oncogenic proteins they code for. Over expression of E6 and E7 maintains the host cells immortalization, regulates cell phenotype and malignancy transformation and the consequent loss of mitosis control [52]. E6 and E7 are highly expressed in tumor cervical cells, alter the synthesis and activity of cytokines and host tumor suppressor proteins, such as p53 [53].

E6 protein contains domain named E6 superfamily and a conserved zinc-binding motif. Through this motif, E6 is able to interfere with the metabolism host proteins such as those related to immune response (TRAF family) [29] and those that control cell cycle, such as p53 [53]. Approximately 46% of E6 structural are helical spanning through 176 amino acid residues and 20% beta-sheet spanning through 79 residues. Figure 3A shows the most stable conformational surface structure of HPV E6 when bound to TRAF5 and their interface of interaction. Figure 3B shows the pattern of alfa-helices and beta-strands that comprise the secondary structure of the protein.

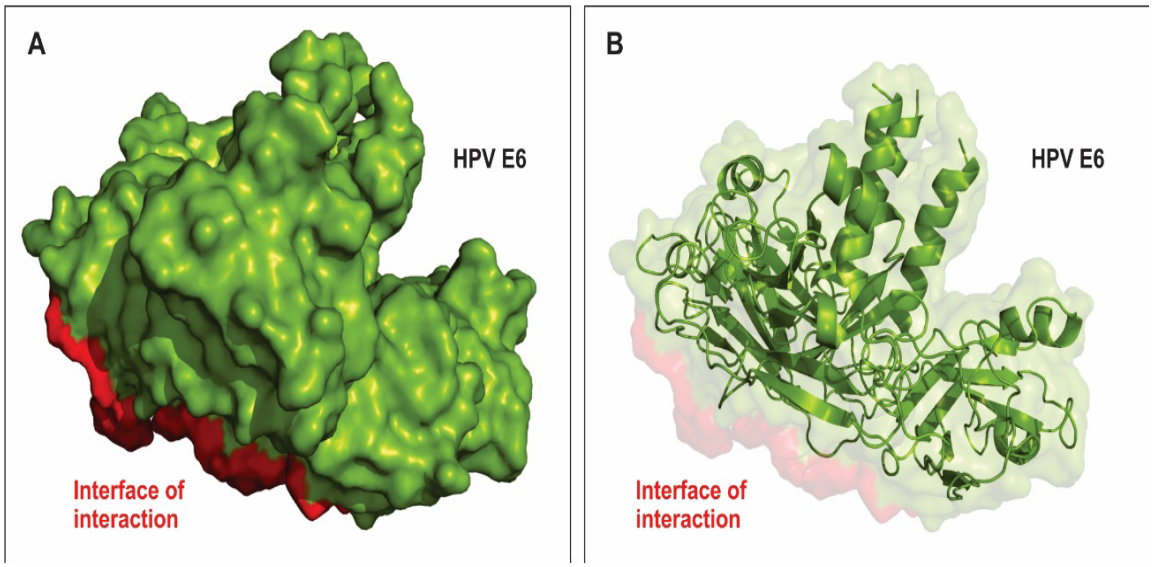

Figure 3: Three-dimensional conformational structure of HPV E6 when bound to TRAF5.

A – Representation of the E6 conformational surface structure. Red amino acid residues show the large interface of interaction between HPV E6 and TRAF5. B – Representation of the secondary structure of E6 with alfa-helices, beta-sheets and the E6 superfamily domain that contains a zinc-finger motif, responsible for the interaction of E6 with host proteins.

Interaction between TRAF5 and HPV E6

Figure 4A and 4B shows the best model of interaction between TRAF5 and HPV E6. Amino acid residues scored as hot spots stabilize the large interface of interaction between the bindings of host-pathogen proteins. The residues that most contribute to free-energy of binding are listed on table 1. The hot spot residues are placed within the interface of interaction (Figure 4) and they interact with amino acid from the other protein through polar, hydrophobic and electrostatic interactions. Interestingly, all the residues that most contribute to the stabilization of the complex are polar amino acids. Polar interactions are key contributors to the specificity of interactions. Here, we hypothesize that for high-risk HPV types (such as HPV 16), the complex formed by E6 and TRAF5 are well stabilized mainly by those residues (Table 1) and this interaction may reduce TRAF5 activity. Therefore, it alters the levels of cytokines for a proper immune response.

Figure 5 shows a small segment of the interface of interaction between the proteins TRAF5 and E6, with the hotspot residues listed on table 1. The amino acid residues Thr81 and Glu79 located on the TRAF5 sequence and the residues Arg427 and Asp502

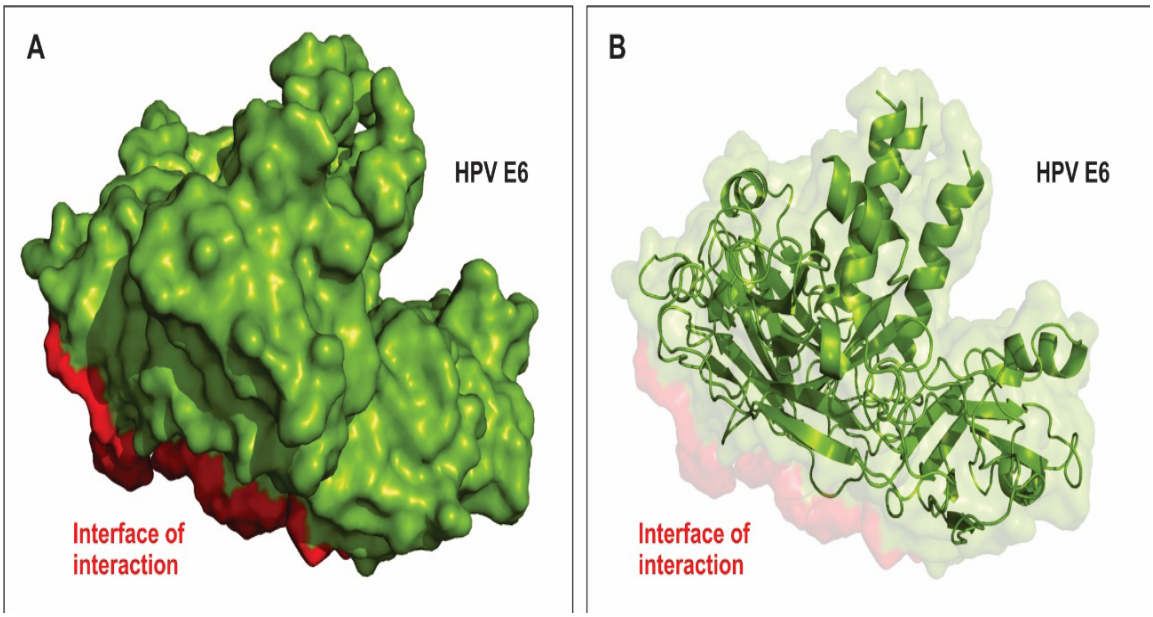

Figure 4: Most stable model of interaction for the complex TRAF5-HPV E6.

A – Representation of the conformational surface structure of the complex. The red region corresponds to the interface of interaction. B – Representation of the secondary structure the complex, which interface between proteins are stabilized mainly by polar interactions.

Protein	Chain	Residue	HP score*
E6	A	Glu79	0.15
E6	A	Thr81	0.13
E6	C	Gln14	0.28
TRAF5	b	Arg427	0.04
TRAF5	c	Arg427	0.08
TRAF5	c	Asp502	0.86

Table 1: Amino acid residues that most contribute to a stable interaction between TRAF5 and E6

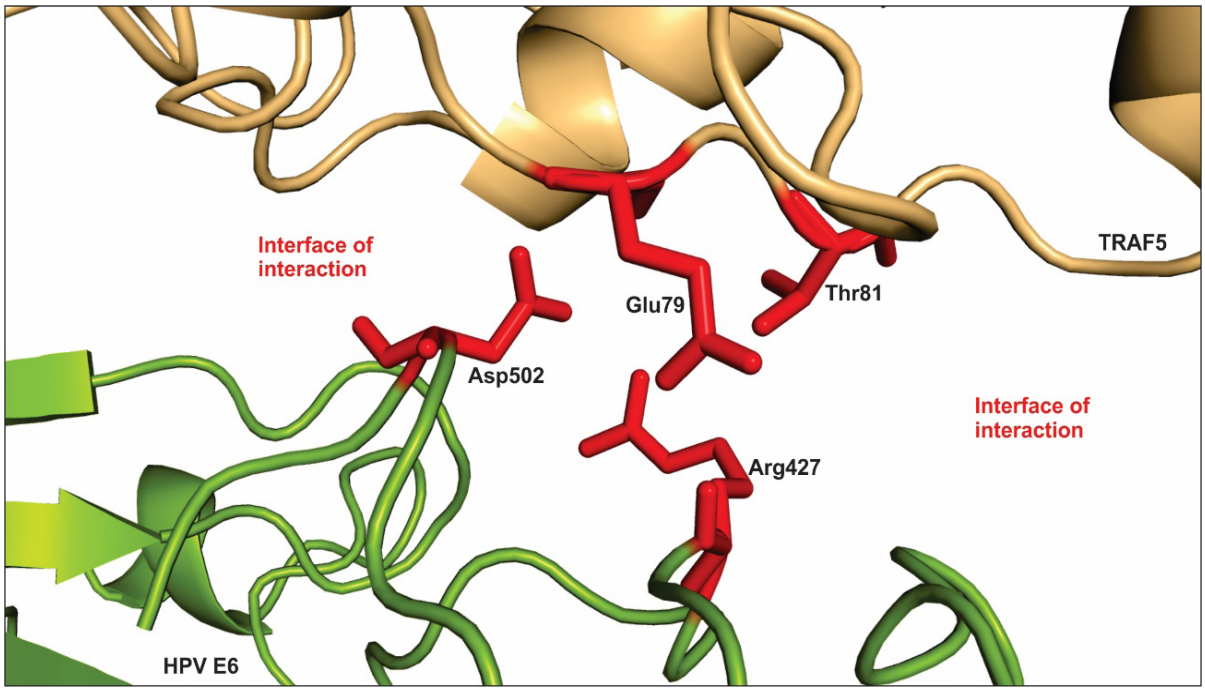

Figure 5: Hot spots within the TRAF5-HPV E6 interface of interaction. The red residues are classified into hot spot due to the contribution that they add to stabilize the complex regarding the free-energy. The green structure is the HPV E6 and the beige structure is the protein TRAF5. The side chains of the residues that participate in the interaction protrude into the interface between the proteins. The neighboring residues also participate in stabilizing the complex but in a lesser extent.

located on the E6 sequence project into the interface of interaction and contribute to the free-energy of the interaction. Neighbor residues also contribute to stabilize the complex. E6 and other oncogenes from high-risk HPV types deregulate immune response and might lead to malignancy transformation of the host cells [52].

The results from our *in silico* approaches showed the most stable model for the interaction between E6 and TRAF5, then we chose a region of the interface of interaction which contains several hot spot residues (Table 1) in order to rationally design a inhibiting peptide against this interaction. Other approaches have designed peptides against HPV [54,55] for the development of vaccines and alternative treatment trials. We designed a peptide containing 21 amino acid residues that interacts with the E6 protein in order to modulate its function and inactivate it (Figure 6).

The rational construction of peptides based on structure explores feasible strategies for new therapies that induce proper immune response in order to combat HPV infections efficiently.

Concluding remarks

HPV is the most common sexual transmitted disease worldwide. It is a complex and expensive public health problem. The disease has a high rate of deaths due to its relation to cancer, principally cervix cancer. Several HPV genes and proteins are potential candidates as genetic markers for the development of cancer and they are target for vaccines and HPV treatment.

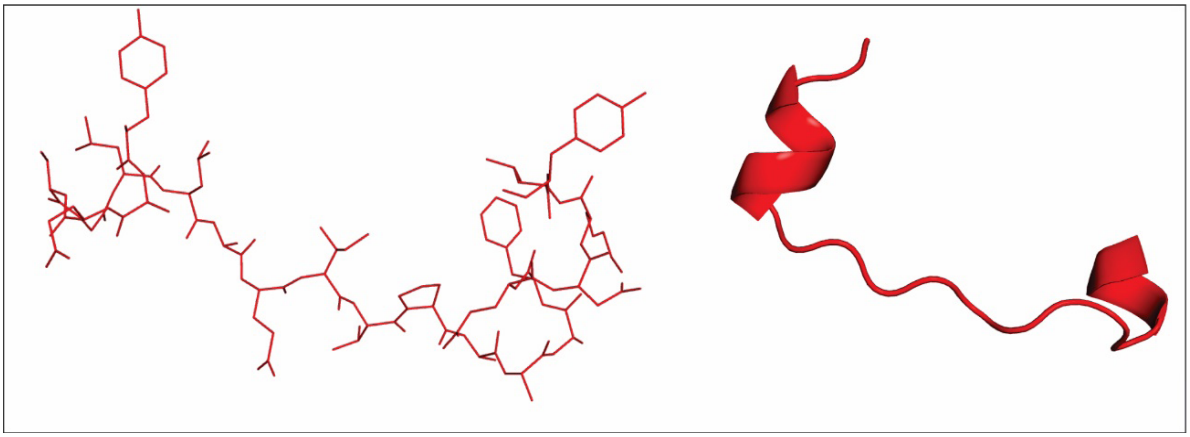

Figure 6: Peptide rationally designed through the screening of hot spots in the interface of interaction between TRAF5-HPV E6. The structure represented on the left is the peptide and its 21 amino acid residue. On the left, the same peptide in its secondary structure representation. The peptide binds to the HPV E6 protein and modulate its function.

Here, we hypothesize that interaction between TRAF5 and E6 could be modulated in order to inhibit the activity of E6. We have shown an *in silico* approach of interaction between TRAF5 and HPV E6 through the identification of hot spots within the interface of interaction of the complex. We propose a new peptide that interacts and inhibits its HPV E6. For a future perspective, the peptide designed will be tested *in vitro* and other regions of the interface of interaction will also be screened so that other peptides could also be designed and tested.

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