Immunoinformatics-Driven Design of an HPV-16 and -18 Synthetic Long Peptide Vaccine

Alexandru TÎRZIU[a,*], Leonard MADA[b], Corina VERNIC[a], and Virgil PĂUNESCU[a, c, d]

[a] Department of Functional Sciences, "Victor Babeş" University of Medicine and Pharmacy, Eftimie Murgu Square, no. 2, 300041 Timişoara, Romania
[b] Syonic SRL, Grigore T Popa Str., no. 81, 300254 Timişoara, Romania
[c] Center for Gene and Cellular Therapies in the Treatment of Cancer Timişoara-OncoGen, Clinical Emergency County Hospital "Pius Brinzeu" Timişoara, Liviu Rebreanu Str, no. 156, 300723 Timişoara, Romania
[d] Immuno-Physiology and Biotechnologies Center, Department of Functional Sciences, "Victor Babeş" University of Medicine and Pharmacy, Eftimie Murgu Square, no. 2, 300041 Timişoara, Romania
E-mails: alexandru.tirziu@umft.ro; leo.mada@syonic.eu; cvernic@umft.ro; vpaunescu@umft.ro

* Author to whom correspondence should be addressed; Tel.: +40 733 733 409

Abstract

Background and Aim: Human papillomavirus types 16 and 18 cause most cervical cancers worldwide. Existing VLP-based vaccines are unable to eliminate ongoing highly oncogenic infections. This study suggests an SLP-based vaccine for already infected patients, even with precancerous lesions. *Methods*: HPV16/18 E6 and E7 proteins' potent HLA class I and class II-restricted epitopes were extracted from IEDB or predicted by ANNs ($IC_{50} < 50$ nM, percentile rank < 2%). Population coverage was assessed via HLA-SLP interactions. SLP constructs, containing specific epitopes linked by cleavable peptides (LMRK, LLSVGG), underwent rigorous screening for allergenicity, toxicity, physicochemical parameters, antigenicity, and diversity. 3D structure prediction employed homology and ab initio modeling and was validated via QMEAN4 score and Ramachandran plots. Molecular docking (HADDOCK-2.4) evaluated SLP-TLR2 interactions. MD studies (GROMACS-2023.1) investigated SLP behavior in an electroneutral aqueous environment (300 K, 1 atm, 100 ns) via RMSD, RMSF, SASA, and R_{gyr} dynamics. *Results*: 28 class I and 16 class II-restricted epitopes had an $IC_{50} < 50$ nM and provided population coverages of 98.18% (class I) and 99.81% (class II). The selected 25 SLPs expressed a mean 1.5×10^{-2} toxicity score, no allergenic properties, a mean instability index of 37.61, and a mean VaxiJen score of 0.97. The QMEAN4 score ([0.665;0.813]), with >90% of the residues in the most favorable regions reflect good quality 3D structures. Molecular docking studies provided a ΔG of -13.20 ± 1.42 kcal/mol, and an average K_d of 3.5×10^{-9} M. MD simulations reflect favorable thermodynamic properties, based on mild RMSD ([0.1;0.87] nm), RMSF, SASA (49.37 ± 1.54 nm^2) and Rgyr ([1.15;1.34]) fluctuations over the 100-ns simulations. *Conclusions*: 25 in silico-designed SLPs stimulate both innate and adaptive immunity, with little to no toxic or allergenic effects. The presented vaccination platform could exert both a therapeutic (by tumor cell clearance) and a prophylactic effect (by eradicating E6 and E7+ premalignant cells).

Keywords: Human papillomavirus; Cervical cancer; Therapeutic vaccine; Molecular modelling; *in silico*; Synthetic long peptides