Journal of Genetic Engineering and Biotechnology 23 (2025) 100485

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

In silico drug repurposing for potential HPV-induced skin wart treatment – A comparative transcriptome analysis

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ARTICLE INFO

Keywords:
Gene expression analysis
HPV
Molecular docking
RNA-sequencing
Skin wart

ABSTRACT

Introduction: Warts are dermal disorders resulting from HPV infection and can be transmitted by direct contact. Existing treatment approaches, such as topical treatment with salicylate, have low efficiency and demonstrate side effects. Thus, the discovery of potent drug treatments for skin warts is necessary. Here we propose the use of alternative medications for the possible treatment of skin warts with the help of comparative transcriptome analysis and drug repurposing approaches.

Methods: Gene expression datasets related to HPV-induced warts and cervical cancer were extracted from the GEO database. Differentially expressed genes (DEGs) were identified using DESeq2 in the Galaxy database. Upregulated DEGs were assessed for druggability using the DGIdb tool. Gene ontology and enrichment analysis were performed to investigate the characteristics of druggable DEGs. A molecular docking virtual screening was conducted using PyRx software to identify potential therapeutic targets for skin warts. The interactions between selected drug candidates and the target protein were analyzed using the BIOVIA Discovery Studio. The physicochemical characteristics of potential pharmaceuticals were evaluated using the SwissADME database. Finally, the molecular dynamics (MD) simulation was performed to validate the stability and dynamic behavior of drug-protein interactions.

Results: Based on the findings from gene expression profiling, Integrin Alpha-X (ITGAX, CD11c) has been identified as a candidate protein that is significantly upregulated in individuals afflicted with skin warts. Integrin Alpha-X plays a crucial role in mediating intercellular interactions during inflammatory processes and notably enhances the adhesion and chemotactic activity of monocytes. Through molecular docking, MD, and physicochemical analyses, it has been demonstrated that dihydroergotamine effectively inhibits the ITGAX protein, suggesting its potential as a therapeutic agent for the management of skin warts.

Conclusion: Dihydroergotamine can be repurposed as a potential drug in the treatment of skin warts by targeting Integrin Alpha-X protein.

1. Introduction

Warts represent benign skin proliferation induced by the human papillomavirus (HPV). These lesions may manifest in diverse anatomical locations, such as the hands, feet, and facial regions, and are distinguished by their coarse texture and elevated morphology. Typical common warts often present as diminutive, granular protuberances that may exhibit a surface reminiscent of cauliflower and can range in color from flesh-toned to slightly darker shades. Warts possess a contagious nature and have the potential to propagate through direct contact or indirectly through contaminated surfaces.[1] Current therapeutic options for cutaneous warts encompass a diverse array of topical and procedural interventions, each possessing unique efficacies and inherent limitations. Topical interventions are frequently commenced with salicylic acid, a keratolytic compound that facilitates the desquamation of epidermal cells infected by warts. Nevertheless, its efficacy is significantly reduced in the presence of larger or more deeply situated warts, and it is contraindicated for sensitive anatomical regions such as the facial area and genitals due to the potential for irritation and chemical burns. Other topical alternatives consist of cantharidin, which could

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https://doi.org/10.1016/j.jgeb.2025.100485
Received 24 November 2024; Received in revised form 12 March 2025; Accepted 23 March 2025

acquire excessive clearance results but might also additionally reason blister and discomfort, and imiquimod, an immune reaction modifier that has proven promise in treating refractory warts, albeit with ability facet results like erythema and ulceration. Procedural remedies consisting of cryotherapy also are prevalent, using liquid nitrogen to freeze warts, usually attaining comparable achievement quotes to topical remedies. While cryotherapy is powerful, it could be painful and might result in complications like blistering or scarring. Additional alternatives consist of intralesional injections of immunotherapy agents, which may be powerful for irritating warts however require cautious patient selection because of various immune responses. Surgical elimination is commonly reserved for instances resistant to different remedies because of its invasive nature and related risks. Overall, even though several remedy modalities exist, their effectiveness can range primarily based totally on patient responses, wart type, and location, necessitating a tailor-made method for management.[2–4]

Human papillomavirus (HPV) is classified as a DNA virus belonging to the *Papillomaviridae* family, which encompasses both low-risk and high-risk variants. The pathogenic potential of the virus varies according to the specific strain, leading to the manifestation of benign cutaneous infections (such as warts) or the development of malignant cervical neoplasms.[5,6] Diseases resulting from HPV infections are highly contagious and are transmitted mainly through direct contact or sexual activities.[7,8] HPV predominantly targets the basal keratinocytes located within the epithelial layers. Nevertheless, HPV virions are unable to traverse intact dermal layers. However, in instances of compromised tissue integrity or following mechanical abrasion, the virus can access the basal layer of the epidermis and keratinocyte cells through the facilitation of alpha integrins, laminins, and annexin A2.[9] In the interim, receptor-mediated endocytosis significantly enhances the mechanism by which viruses penetrate target cells. Integrins, characterized as transmembrane glycoproteins, have been shown in research to be instrumental in the infiltration of HPV into keratinocyte cells.[10] The function of integrins is to facilitate cell–cell and cell-extracellular matrix (ECM) linkage. They are also involved in signal transduction into cells.[11,12] So, it is plausible to prevent the manifestation of inflammatory symptoms associated with warts through the inhibition of the integrins implicated in their pathogenesis. Warts, scientifically referred to as papillomas, are categorized as benign neoplasms. These lesions arise from the hyperproliferation of epidermal layers in regions affected by HPV infection.[13] Therefore, by inhibiting the proteins involved in virus penetration as well as the proteins forming the wart, it is possible to cure patients with HPV-induced warts.

In this study, we conducted a differential gene expression analysis (DEG) aimed at identifying biomarker genes associated with warts. Through a comparative analysis of DEGs against a control group, we discerned multiple genes exhibiting either decreased or increased expression levels. Subsequently, to substantiate that the identified DEGs

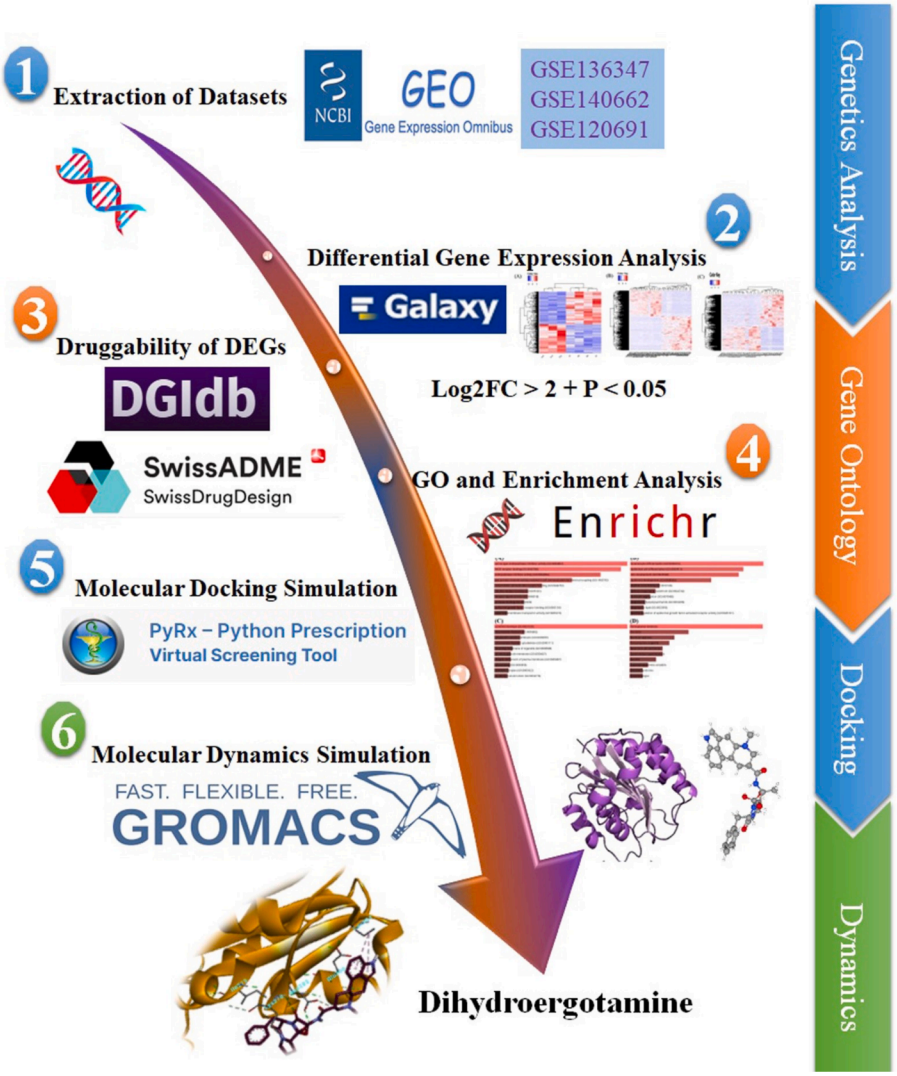


Fig. 1. Comprehensive workflow demonstrating the methodological steps undertaken in this study.

are unequivocally attributable to human papillomavirus (HPV) and that extraneous variables are not implicated, we compared DEGs from male subjects with those from female patients diagnosed with HPV-induced cervical carcinoma, as well as from male individuals suffering from HPV-induced genital warts in the penile region. This methodological approach enabled us to isolate up-regulated genes that concurrently elicit the manifestations of cutaneous warts while also demonstrating elevated expression in cervical cancer. In the subsequent phase, we concentrated on one of the candidate protein products derived from these up-regulated genes to explore the potential repurposing of FDA-approved pharmacological agents for the possible treatment of both skin and genital warts. Fig. 1 demonstrates the workflow and methodological steps undertaken in this study.

2. Methods

2.1. Extraction of gene expression datasets

The primary objective of this study was to identify differentially expressed genes (DEGs) that are significant in the context of skin warts; therefore, datasets were selected through a comprehensive search of the GEO (Gene Expression Omnibus) database utilizing the keywords "wart," "HPV," and "HPV-induced wart" at https://www.ncbi.nlm.nih.gov/geo/.[14] Thus, the criteria for inclusion and exclusion were defined as samples obtained from individuals with HPV-induced skin and cutaneous warts who had not undergone any prior therapeutic interventions, alongside datasets that included normal control samples, respectively (GSE136347 and GSE140662). Since the datasets concerning skin warts were sourced exclusively from male participants, a search was conducted in the GEO database for cervical cancer samples linked to HPV infection to standardize the data and reduce any possible sexual bias, as well as to ascertain that the identified DEGs were indeed a consequence of HPV infection. At this point, only samples obtained from the whole blood of female patients diagnosed with HPV-induced cervical cancer who did not receive any form of treatment were incorporated into the study (GSE120691).

2.2. Dataset quality check and optimization

Upon acquisition of the raw sequencing datasets from the Gene Expression Omnibus Sequence Read Archive (GEO SRA), these datasets were subsequently uploaded to the Galaxy Online Workflow utilizing the SRA server tool at https://usegalaxy.eu/.[15] Thereafter, an examination of dataset quality control was conducted employing the FastQC tool (V. 0.74).[16] At this point, parameters such as per base sequence quality, per base GC content, sequence length distribution, and levels of sequence duplication were thoroughly analyzed. In instances where data quality was believed suboptimal, the datasets underwent trimming and optimization processes using Trim Galore (V. 0.6.7). This tool effectively removes sequencing adapters and eliminates datasets that exhibit low quality.[17]

2.3. Differentially gene expression analysis

To determine the genes that were called, the optimized sequencing datasets were aligned with the human reference genome version GRCh38, employing the HISAT2 alignment tool version 2.2.1.[18] Genes exhibiting a normalized count of less than 20 were excluded to remove the occurrence of false positive results. Subsequently, to identify differentially expressed genes (DEGs), the expression profiles of disease samples (HPV positive) were compared with those of control samples utilizing the DESeq2 package version 2.11.40.8.[19] This software conducts an analysis based on negative binomial distribution to assess the gene fold change within high-throughput sequencing datasets. In this context, genes demonstrating a statistically significant expression variation exceeding 2-fold (Log2FC $>$ 2) along with an Adjusted P-value of less than 0.05 were selected. Among the DEGs, those exhibiting elevated expression levels and commonality across all three patient cohorts (individuals with HPV-induced warts and cervical cancer) were chosen for further analysis.

2.4. Examining the druggability of upregulated DEGs

Upregulated DEGs were examined to identify those whose protein products have the potential to be targeted by drugs. To facilitate this analysis, the Drug-Gene Interaction database (DGIdb; https://dgidb.org/) was employed to evaluate the druggability of the identified DEGs. This database identifies drug-gene interactions with a high degree of precision through the inspection of a multitude of resources, including Drug Bank.[20] Here we retrieved upregulated DEGs from skin wart datasets that are identified as targets for drug discovery.

2.5. Gene ontology and enrichment analysis

The characteristics of druggable DEGs, including function, expressing tissue, and subcellular location (i.e., gene ontology (GO)), were systematically investigated utilizing the EnrichR, KEGG, and GeneCards (V. 5.14) databases.[21–24] The outcomes with an enriched P-value lower than 0.5 and the highest combined score were selected for further analysis. Furthermore, a heatmap was constructed to elucidate the genes exhibiting dysregulation, employing normalized read counts of genes with a P-value less than 0.05.

2.6. Virtual molecular docking simulation

In the next, a protein that possessed all requisite attributes to be identified as the etiological factor for skin warts in individuals afflicted with HPV was designated as the prospective molecular target for potential therapeutic intervention against skin warts. The three-dimensional molecular configuration of the selected protein target was obtained from the SMR (Swiss-Model Repository; https://swissmodel.expasy.org/repository) database in PDB format.[25] The integrity of the acquired protein structure was subsequently refined using UCSF Chimera version 1.16.[26] The chemical structure of FDA-approved pharmaceuticals was retrieved from the Zinc database in SDF format (https://zinc.docking.org/).[27] Subsequently, molecular docking procedures were conducted utilizing the PyRx software version 0.8. This computational tool employs AutoDock Vina for the virtual simulation of docking interactions.[28] Pharmaceuticals exhibiting a binding affinity exceeding −9 kcal/mol were regarded as ideal candidates for the inhibition of the target protein based on prior studies and empirical observations.[29,30] The BIOVIA Discovery Studio Visualizer (V. 21.1.0.20298) was utilized to analyze and illustrate the interactions between the drug and the protein.[31] At this point, the SwissADME database (http://www.swissadme.ch/) was consulted to assess the physicochemical characteristics of potential pharmaceuticals for the probable treatment of skin warts.[32].

2.7. Molecular dynamics simulation

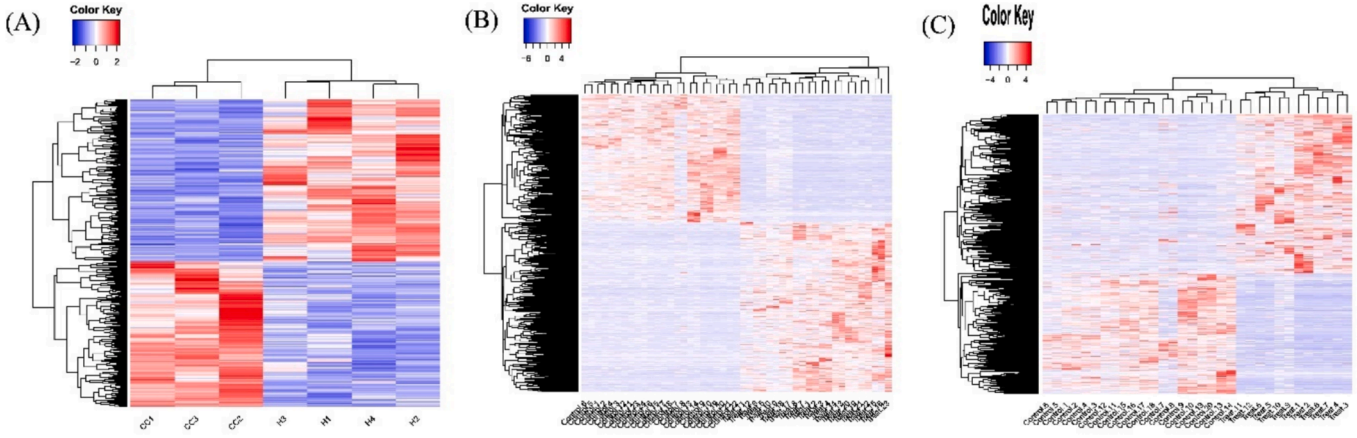
The virtual molecular docking results were subjected to molecular dynamics (MD) simulations using GROMACS version 2021.1. The CHARMM force field was selected to evaluate the stability of the drug-protein complex. The topology files for the best-performing ligand were generated using the Swiss Param server (http://swissparam.ch/).[33] The drug-protein complex was positioned within a cubic box, maintaining a distance of 1 nm from all edges. The simulation box was then solvated with SPC water molecules, and then sodium and chloride ions were added to neutralize the system. Energy minimization was performed using 5000 steps of the steepest descent algorithm, followed by a two-phase equilibration process: first under NVT (constant number of particles, volume, and temperature) conditions at 300 K for 100 ps, and

then under NPT (constant number of particles, pressure, and temperature) conditions at 300 K and 1.0 bar for 100 ps. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method with a 1.0 nm cutoff and 0.16 nm grid spacing. After reaching equilibrium, a 100 ns (ns) MD simulation was performed and the stability of the system was evaluated by analyzing the root mean square fluctuation (RMSF), root mean square deviation (RMSD), and radius of gyration (Rg).[34]

3. Results

3.1. Dataset extraction, optimization, and validation

We identified three distinct datasets including 1) GSE136347 (MHW): this dataset includes samples derived from male subjects presenting with HPV-induced cutaneous warts as the core dataset for skin warts. Here biopsy specimens were collected from the hand and forearm, encompassing 24 samples from healthy controls and 23 samples affected by skin warts.[35] 2) GSE140662 (MGW): this dataset pertains to cutaneous warts localized to the genital region, specifically isolated from the penile area used as an affirmative dataset for skin warts. The MGW



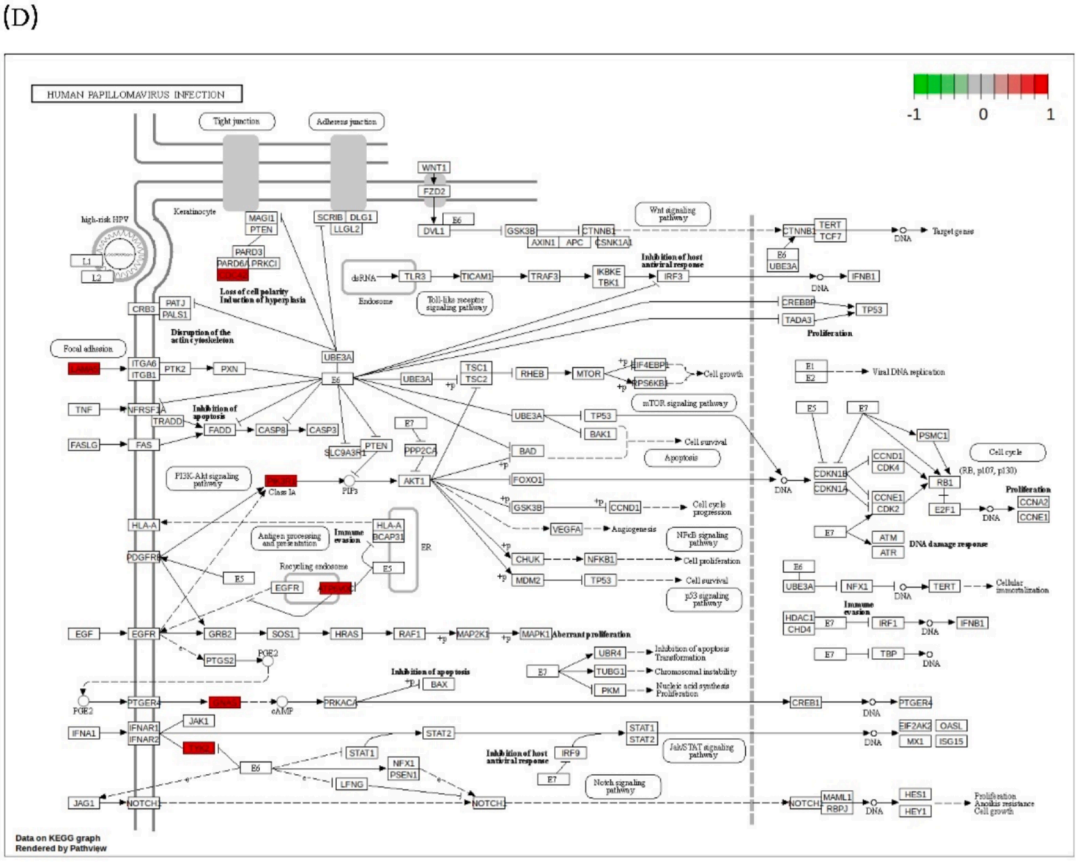


Fig. 2. Genes with variable expression and related molecular pathways involved in HPV infection. A) Heatmap demonstrating DEGs in MHW; B) MGW; and C) FCC samples from patients with HPV infection. D) Molecular pathways involved in HPV infection retrieved from the KEGG database.[22,24]

dataset consists of three normal samples and four samples from patients exhibiting genital warts, which were obtained from keratinocyte biopsies.[36] 3) GSE120691 (FCC): this dataset encompasses gene expression profiles of females diagnosed with HPV-induced cervical carcinoma, intended for normalization purposes, and diminishing potential sexual bias. The FCC dataset includes 12 samples of cervical cancer and 20 samples from normal controls.[37] To identify DEGs, a comparative analysis was conducted between patient samples and their corresponding controls. Throughout the processes of dataset refinement and optimization, the Trim Galore tool enabled the restoration of datasets with an elevated Phred quality score of 40. After the alignment of trimmed datasets with the human reference genome, HISAT2 effectively allocated approximately 70 % of genes to their appropriate loci within the human genome.

3.2. About 334 upregulated DEGs were discovered in skin warts

Following the identification of DEGs within each group through the comparative analysis of pathological and non-pathological subjects, a total of 662 shared DEGs were discerned among males presenting with skin warts (both dermal and genital specimens; Supplementary Table 1). In this context, 334 DEGs were upregulated, whereas 328 DEGs were downregulated when compared with control subjects (Adjusted P-value < 0.05). A comprehensive examination of the upregulated DEGs yielded the identification of 175 druggable genes, as per the findings derived from DGIdb (Supplementary Table 2).[20] In women afflicted with HPV-induced cervical carcinoma (FCC), a total of 548 DEGs were discerned. Among these, 291 genes exhibited upregulation, while 257 genes demonstrated downregulation when compared with their corresponding control samples (Supplementary Table 3). Through a comparative analysis of the upregulated DEGs in men presenting with cutaneous warts and females diagnosed with cervical cancer, two shared genes; *c6orf62* (Chromosome 6 Open Reading Frame 62) and *itgax* (Integrin Alpha-X, or CD11c) were identified. This observation implies that despite the distinct etiological HPV variants involved, they may elicit the activation of an identical genomic signature (Fig. 2A, B, and C).

3.3. Upregulated genes control keratinocyte differentiation and keratosis

Gene ontology analysis was conducted on 334 upregulated genes, revealing their significant association with biological processes related to keratinocyte and epidermal cell differentiation, leukocyte aggregation, and skin development. The molecular functions that exhibited the highest enrichment included gap junction channel activity involved in cell communication and RAGE receptor signaling, as illustrated in Fig. 3A. These genes are predominantly found within the cellular components of the cornified envelope and the intermediate filament. From a phenotypic perspective, the upregulated genes are associated with impaired skin barrier function and hyperkeratosis. Notably, these genes are also implicated in palmoplantar keratosis, a condition characterized by excessive thickening of the epidermis (P-value < 0.05; Fig. 3B). Given that Integrin Alpha-X was highly upregulated in subjects with HPV-induced skin warts and was also common to both individuals with skin warts and patients with HPV-induced cervical cancer and showed significant enrichment in pathological conditions such as inflammation and monocyte recruitment, it was selected as a potential candidate for the treatment of skin warts among the upregulated genes. This gene functions as a cell–cell mediator, facilitating the transmission of inflammatory signals (Table 1). Through its role in inflammation, the Integrin Alpha-X protein recruits monocytes to the site of infection. Furthermore, integrins are recognized as critical modulators of HPV entry into keratinocyte cells. The molecular pathways and genes associated with HPV infection are illustrated in Fig. 2D.

3.4. Dihydroergotamine might cure skin warts

After pinpointing Integrin Alpha-X as the candidate protein, the 3D structure of Integrin Alpha-X was obtained from SMR and subsequently inputted into the virtual screening and molecular docking procedure

Table 1
The properties of two upregulated DEGs in MHW, MGW, and FCC patients.

Gene name	Fold Change in Skin warts	Fold Change in Cervical cancer	Cellular location	Molecular Function
itgax	2.8	0.84	Cytoplasm and cytosol	cell–cell interaction, signal transduction
c6orf62	2.0	1.3	Cytosol and nucleus	Unknown

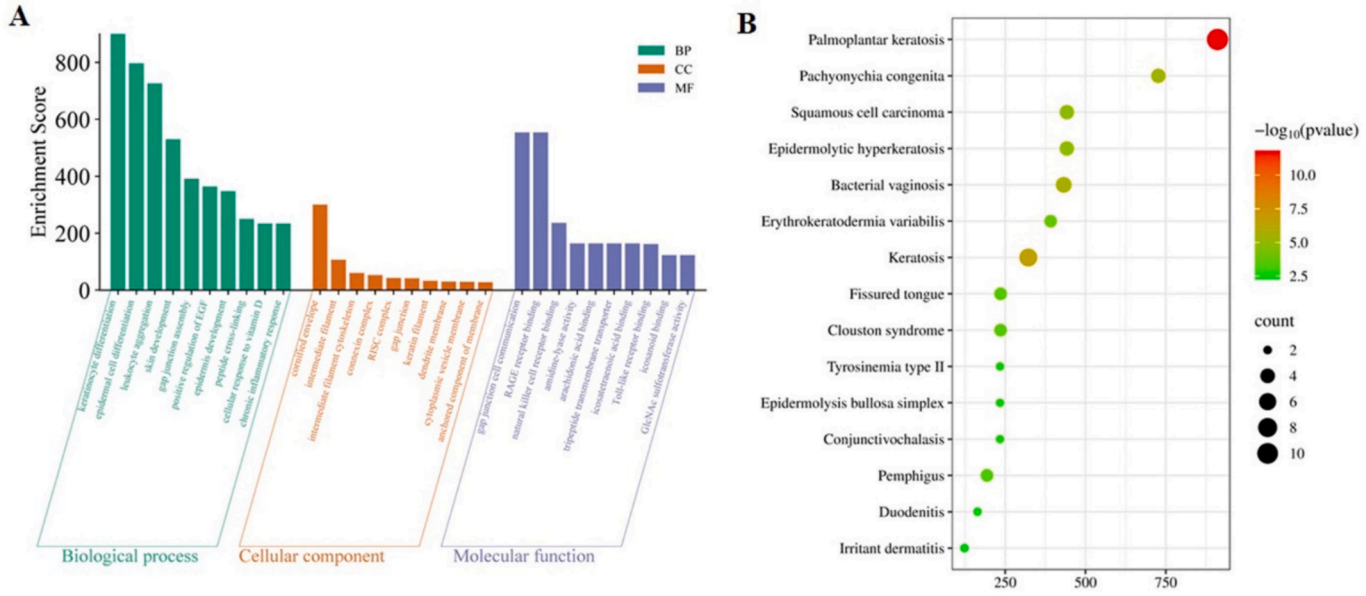


Fig. 3. GO properties of upregulated DEGs. (A) Biological Process (BP), Cellular Component (CC), Molecular function (MF); and (B) Related diseases sorted by enrichment score.[38]

following optimization. Through the docking process, we identified five potential drugs; digoxin, velpatasvir, irinotecan, dihydroergotamine, and venetoclax; all exhibiting a binding affinity greater than −9 kcal/mol, suggesting they could effectively inhibit ITGAX (Table 2). Notably, only dihydroergotamine displayed favorable pharmacokinetic characteristics, making it a strong candidate for treating HPV-induced skin warts. Dihydroergotamine formed three hydrogen bonds and six Van der Waals interactions with the ITGAX protein (Fig. 4 and Supplementary Table 4).

3.5. Molecular dynamics confirm the results

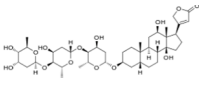
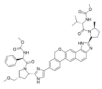
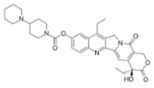
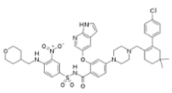
To evaluate the stability of the dihydroergotamine (DHE)- Integrin Alpha-X (ITGAX) complex, molecular dynamics simulations were performed up to 100 ns. MD simulation results indicate a stable system. The average RMSD was 0.17 nm (nm), demonstrating that the DHE-ITGAX complex remains stable during simulation. The RMSD measures the deviation of the atomic positions of the protein from the reference structure.[39] We also measured the stability of the compact structure. The compactness of the protein structure was ensured by a low average value of Rg (1.53 nm) and very low fluctuations (less than 0.1 nm) of the stable trajectory. The RMSF value for the amino acids involved in the interaction, including residues Asp268 (0.1574 nm), Tyr269 (0.0744 nm), Lys270 (0.1665 nm), Ile273 (0.1074 nm), Glu300 (0.1137 nm), and Asp303 (0.0935 nm) indicate that the fluctuations of individual residues or regions of the protein are within the expected range (Fig. 5).

4. Discussion

Human papillomavirus (HPV) causes diverse clinical manifestations and malignancy in people. Certain HPV strains do not show any symptoms, while others result in skin warts or malignancies, such as cervical cancer. At present, the most effective method for addressing HPV infections is to provide vaccinations at a younger age. Nevertheless, there is currently no established treatment to eradicate the virus in patients. In this research, we aimed to identify a specific drug that would target the agent responsible for skin warts in individuals affected by HPV. To achieve this, we analyzed three datasets, including GSE136347 (MHW), GSE140662 (MGW), and GSE120691 (FCC), to pinpoint the genes associated with the development of skin warts. The MHW dataset was associated with male patients presenting with skin warts collected from the hand and forearm.[35] MGW was also correlated to male patients with skin warts in the genital area.[36] Through gene expression analysis, it was discovered that there are 662 genes exhibiting notable semantic differences between patients with skin warts and the control group. Of these, 334 DEGs showed an increase in expression, while 328 DEGs demonstrated a decrease when compared to the control. Furthermore, an evaluation of the druggability of the genes revealed that the protein products of 175 upregulated DEGs can be targeted by drugs approved by the FDA. To normalize the data, reduce gender bias, and confirm that HPV infection is the primary cause of the observed changes in gene expression, the dataset regarding women with HPV-induced cervical cancer, accession number GSE120691 (FCC), was also employed.[37] In FCC, 548 DEGs were found, of which 291 had increased and 257 had decreased expression compared to the control. Through the analysis of upregulated DEGs across the three datasets, two shared upregulated DEGs, *c6orf62* and *itgax* were discovered.

The findings from gene ontology analyses indicate that the upregulated DEGs are vital in gap junction channel activity involved in cell communication and RAGE receptor signaling. The latter plays an important role in inflammation.[40] These genes are also involved in keratinocyte differentiation and proliferation, as well as in diseases such as palmar keratosis, in which the skin becomes thickened and inflamed (like a wart on the skin). Among the candidate genes, *itgax* (Integrin Alpha-X) had an increased expression of 2.8 in wart lesions. This gene is involved in neutrophil degranulation, cellular response to cytokine stimulation, neutrophil activation associated with immune responses, and biological processes of neutrophil-mediated immunity (according to EnrichR). The 1163 amino acid Integrin Alpha-X protein is located on the cytoplasmic vesicle membrane, the intermediate filament cytoskeleton, the organelle-associated membrane, and the secretory granule membrane.[21] This protein mediates cell–cell interaction in inflammatory responses and particularly facilitates monocyte adhesion and chemotaxis.[11] Integrin Alpha-X also allows neutrophils and monocytes to adhere to stimulated endothelial cells. It is also involved in the phagocytosis of complement-coated vesicles. Several studies have shown that Integrin Alpha-X is also involved in the development of cancers.[41,42] Given that Integrin Alpha-X protein was highly upregulated in subjects with HPV-induced skin warts and common to both individuals with skin warts and patients with HPV-induced cervical cancer, its protein product was identified as druggable; furthermore, it showed significant enrichment in pathological conditions such as inflammation and monocyte summoning and has a significant role in inflammation and cancer development, the Integrin Alpha-X protein was

Table 2
List of drugs repurposed against ITGAX with a binding affinity greater than −9 kcal/mol.

Drug	Structure	Zinc ID	Binding affinity (kcal/mol)	Prescription
Dihydroergotamine		ZINC3978005	−9.1	Migraine
Digoxin		ZINC242548690	−9.6	Atrial fibrillation
Velpatasvir		ZINC203686879	−9.4	Hepatitis C infection
Irinotecan		ZINC1612996	−9.1	Rectal cancer
Venetoclax		ZINC150338755	−9.1	Lymphocytic leukemia

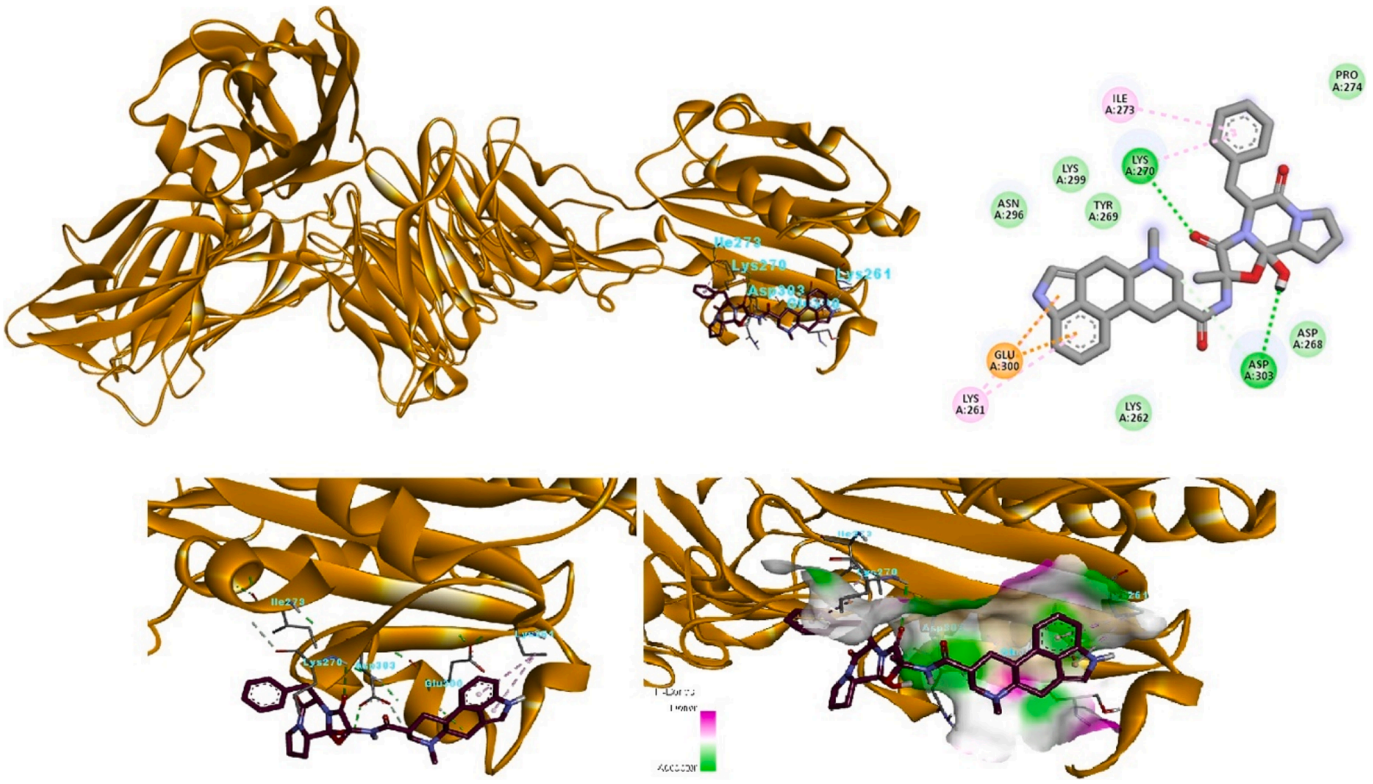


Fig. 4. Attachment of dihydroergotamine drug in the active site of Integrin Alpha-X protein and inhibition of its activity.

selected as a potential target for treating HPV-induced skin warts. Therefore, the Integrin Alpha-X protein entered the virtual molecular docking and drug design process. Five potential drugs with binding affinities greater than −9 kcal/mol were found to be able to inhibit the protein. Only dihydroergotamine had acceptable drug potency and ADME profile to be selected as a potential drug for the treatment of cutaneous warts. Dihydroergotamine is an alkaloid prescribed for the treatment of migraine headaches.[43] This drug performs its anti-migraine activity by an agonistic effect on the serotonin 5-HT2B receptor.[44,45] We then evaluated the stability of the dihydroergotamine in interaction with the Integrin Alpha-X protein using MD simulations. A low and stable RMSD indicates that the DHE-ITGAX complex maintains its overall fold and does not undergo large structural changes. Also, a stable Rg value indicates that the structure does not significantly expand or collapse during the simulation and maintains a fixed overall shape. However, stable RMSD (0.17 nm) and Rg (1.53 nm) values indicate that the DHE-ITGAX complex maintains its structural integrity and compactness throughout the simulation process. Moreover, consistent RMSF values, particularly for amino acids involved in receptor-ligand interactions, suggest that this specific region of the protein remains stable throughout the simulation.

Intending to treat skin warts, Toh et al. designed an active antibody against skin warts with the help of *in silico* bioinformatics methods. Although this antibody has a good efficiency in treatment (more than 90 %), its design and manufacture require a lot of money compared to chemical drugs.[46] Chadha et al. proposed immunotherapy using diphencyprone to treat patients with HPV-induced skin warts who did not respond to cryotherapy.[47] In one study, researchers treated skin warts through hyperthermia and studied their gene expression variation. They found that hyperthermia suppresses proteins responsible for keratocyte division and differentiation.[48] Cryotherapy, which freezes wart tissue with liquid nitrogen, is quick, minimally invasive, and has a high success rate for wart removal, but may need multiple sessions, can cause pain and blistering, and does not address the underlying HPV infection.[49] Immunotherapy, which uses the immune system to target and destroy wart tissue, can be effective for refractory warts and may provide longer-lasting results, but can cause local skin reactions, requires multiple treatments, and its efficacy varies among individuals.[50] Hyperthermia, on the other hand, applies heat to wart tissue, can inhibit keratinocyte division and differentiation, reducing wart size and potentially enhancing other treatments, but requires specialized equipment, can cause discomfort, and its long-term efficacy is still under investigation.[51] These studies suggest that there is no definitive cure for cutaneous warts and that inhibition of cell-to-cell communication and keratinocyte differentiation may provide suitable treatment options. Meanwhile, we have not found any studies like this that attempt to find and repurpose FDA-approved drugs for the treatment of HPV-induced skin warts employing systems biology and bioinformatics tools. We believe that suitable treatment options can be found using the methods used in this study, in which data from individuals with skin warts and cervical cancer are studied simultaneously using systems biology and bioinformatics rapidly and cost-effectively. However, until the results obtained in this study and the selected drug are validated *in vitro* and *in vivo*, these results cannot be accepted with certainty. On the other hand, we encountered several limitations in this study. We did not find high-throughput sequencing data from women with warts that could be used for genetic analysis. Additionally, larger gene expression datasets may have yielded better results. Thus, we highly recommend conducting genomics and proteomics studies on skin warts in women. For females, the primary focus has been on HPV for the prevention or treatment of cervical cancers rather than skin warts, which is a contrast to the focus in males. However, this study holds potential insights, as the targets may be sex-dependent. It's crucial to account for and eliminate sex bias in any genomic or proteomic screening.

5. Conclusion

In the present study, we identified the Integrin Alpha-X protein, which appears to play a significant role in the pathogenesis of skin warts through the application of *in silico* methodologies and systems biology

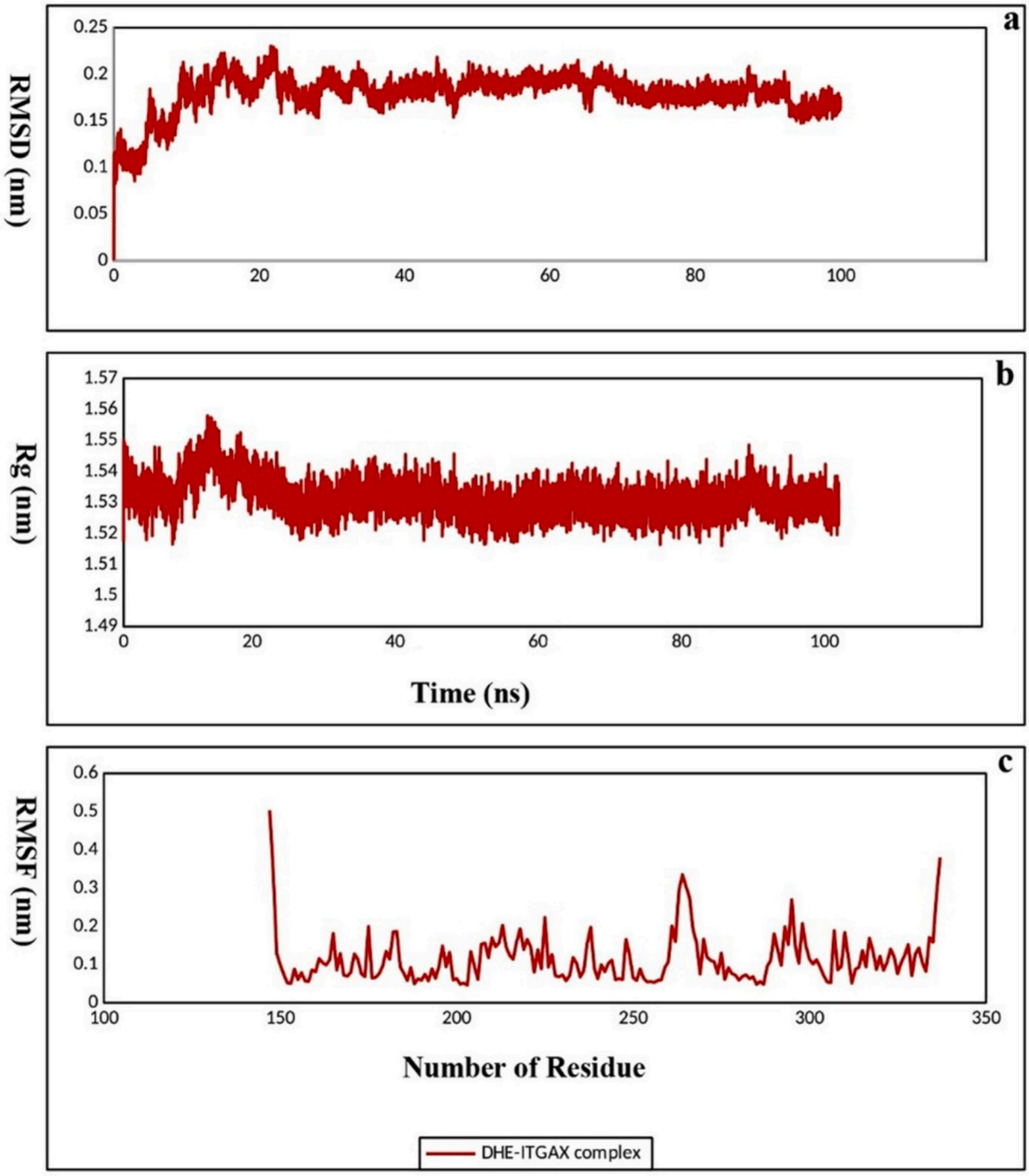


Fig. 5. Results of MD simulation between DHE-ITGAX complex. (A) RMSD; (B) Rg; and (C) RMSF results of the complex.

approaches. Furthermore, through the process of molecular docking and simulation, we successfully identified and proposed dihydroergotamine as a therapeutic agent for this condition by specifically targeting the Integrin Alpha-X protein. This drug has FDA approval and may be administered in the management of skin warts following the completion of investigations concerning its efficacy both *in vitro* and *in vivo*.

Statements & declarations

Availability of data and material

The data used in this study will be made available upon request. The datasets GSE136347, GSE140662, and GSE120691 were acquired from the GEO database at https://www.ncbi.nlm.nih.gov/geo/.

CRediT authorship contribution statement

Navid Kashani: Formal analysis. **Amir Sabbaghian:** Investigation, Formal analysis, Conceptualization. **Khadijeh Ahmadi:** Investigation. **Mahdi Aalikhani:** Methodology, Investigation, Data curation, Conceptualization.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Funding

This study did not receive financial support.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgeb.2025.100485.

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