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Identification of proteins' expression pathway and the effective miRNAs for the treatment of human papillomavirus-induced cervical cancer: in-silico analyses-experimental research

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Introduction: Cervical cancer is the fourth most common cancer in women. The risk factors for cervical cancer include human papillomavirus (HPV) infection, age, smoking, number of pregnancies, use of oral contraceptives, and diet. However, long-term HPV infection appears to be the main risk factor for developing cervical cancer. This in-silico analysis aims to identify the expression network of proteins and the miRNAs that play a role in the development of HPV-induced cervical cancer.

Methods: The critical proteins and miRNAs were extracted using the DisGeNET and miRBase databases. String and Gephi were applied to the network analysis. The GTEx web tool was utilized to Identify tissue expression levels. The Enrichr website was used to explore the molecular function and pathways of found genes.

Results: Ten proteins, TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN, were identified as the most critical shared gene network among cervical cancer and HPV. Seven miRNAs were found, including hsa-mir-146a, hsa-mir-27, hsa-mir-203, hsa-mir-126, hsa-mir-145, hsa-mir-944, and hsa-mir-93, which have a common expression in cervical cancer and HPV.

Conclusion: Overall, the gene network, including TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN, and Also, hsa-mir-145, hsa-mir-93, hsa-mir-203, and hsa-mir-126 can be regarded as a gene expression pathway in HPV-induced cervical cancer.

Keywords: cervical cancer, gene expressions, gene network, human papillomavirus, MicroRNAs

Introduction

Cervical cancer ranks as the fourth most common cancer in women and is a significant cause of cancer-related deaths. Around 85% of cervical cancer deaths worldwide occur in underdeveloped or developing countries. Mortality rates in low- and middle-income countries are 18 times higher than in wealthier countries[1]. The risk factors for cervical cancer include human papillomavirus (HPV) infection, age, smoking, childbirth, oral contraceptive use, and diet[2]. Long-term HPV infection is the primary risk factor for cervical cancer[3]. In other phrases, cervical cancer develops from normal cervical epithelium through the progressive development of low-grade and high-grade cervical intraepithelial lesions (CINs), with HPV (serotypes 16 and 18) infection playing a significant role[4].

Non-coding (nc) RNAs are RNA molecules that is transcribed from DNA but not translated into protein. Micro(mi)RNAs are an important subgroup of the non-coding RNA family, typically consisting of 19–25 nucleotides. Most miRNAs have highly conserved sequences and play a role in various cellular functions

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through post-transcriptional regulation[5]. Studies have provided evidence that several miRNAs are associated with the development of cervical cancer[6]. Furthermore, miRNA can impact the replication of HPV DNA. This can lead to pathways such as abnormal hypermethylation of other miRNAs and resistance to drugs. As a result, it can play a significant role in the development and poor prognosis of cervical cancer caused by HPV infection[7].

Disruption in miRNA synthesis during viral infection, processing machinery, or the expression of specific miRNAs can impair cellular function, potentially leading to pathological conditions such as cancer[8]. In this regard, Herpesviruses establish stable latency programs, including Kaposi sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV), expressing only a minimal subset of their protein-coding open reading frame (ORF)s. These viruses can evade immune detection by relying on viral miRNAs instead of proteins. Viral miRNAs influence numerous cellular pathways without producing foreign protein antigens that could provoke an immune response. During KSHV and EBV infections, viral miRNAs are involved in mechanisms affecting cell survival, proliferation, and, ultimately, oncogenesis[9].

Additionally, viruses can disrupt host miRNAs. For example, the expression of the human papillomavirus (HPV) E6 or E7 oncogenes upregulates a cluster of host miRNAs that promote the growth of HPV-positive cancer cells by regulating cell proliferation, senescence, and apoptosis[10]. The human T-lymphotropic virus 1 (HTLV-1) essential zipper factor (HBZ) protein activates oncogenic miRNAs miR-17 and miR-21, fostering genetic instability and abnormal cell proliferation[11]. Conversely, hepatitis C virus (HCV) genomic RNA sequesters the liver-specific miRNA miR-122 for viral RNA stabilization and replication, preventing its binding to cellular mRNAs. As miR-122 has a tumor suppressor function, its sequestration by HCV genomic RNA and the resulting derepression of average host oncogenic targets of miR-122 may contribute to oncogenesis during chronic HCV infection[12].

In their current bioinformatics study, the authors aim to identify the protein expression network and the effective miRNAs involved in the development of HPV-induced cervical cancer. They plan to do this through in-silico analysis in order to predict and suggest an effective therapeutic target for treating and preventing the disease.

HIGHLIGHTS

- Ten proteins, TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN, were identified as the most critical shared gene network among cervical cancer and HPV.
- Seven miRNAs were found, including hsa-mir-146a, hsa-mir-27, hsa-mir-203, hsa-mir-126, hsa-mir-145, hsa-mir-944, and hsa-mir-93, which have a common expression in cervical cancer and HPV.
- Overall, the gene network, including TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN, and Also, hsa-mir-145, hsa-mir-93, hsa-mir-27, hsa-mir-203, and hsa-mir-126 can be regarded as a gene expression pathway in HPV-induced cervical cancer.

Methods

DisGeNET

The current study used the DisGeNET database (https://www.disgenet.org/) to identify genes related to cervical cancer and HPV viral infection. DisGeNET is an online platform designed to answer various questions about the genetic background of human diseases. Also, this website is currently one of the largest and most comprehensive repositories of human gene-disease associations[13]. As a query, the keywords cervical cancer and HPV were searched separately. Also, the gene sets related to different stages of cervical cancer disease and oropharyngeal HPV infections were excluded from the study. Finally, two gene sets were obtained. However, DisGeNET may faces several limitations, including issues with data quality due to inconsistencies and varying quality from different sources, and incomplete coverage, missing some known gene-disease associations. Also, this database shows no changes in gene expression[14,15].

Calculate and draw custom Venn diagrams

The website Calculate and draw custom Venn diagrams (https://bioinformatics.psb.ugent.be/webtools/Venn/) was used to find cervical cancer and HPV shared genes. The website has a capable artificial intelligence (AI) for data sharing and graphical representation of associations, intersections, and differences between multiple datasets[16].

miRbase

miRBase database (https://www.mirbase.org/) was used to identify microRNAs. miRBase is the primary public repository and online resource for microRNA sequences and annotations. miRBase has been determining gene names for new microRNA discoveries since its establishment[17]. Although miRBase is highly valued by researchers studying microRNAs there are areas that require improvement, such, as the quality of annotations, data coverage, functional information and the manual verification process[17].

String

In the present study, the string database (https://string-db.org/) was used to identify the gene network and extract critical gene hubs. The string is a biological database and web resource of known and predicted protein-protein interactions (PPI); and contains data from multiple sources, including experimental data, computational prediction methods, and public text collections[18]. Also, to find critical gene hubs, after searching for shared genes as a query, based on the type of nodes interaction, unimportant items were removed, and finally, a network with a very high concentration was achieved. The STRING database does have some limitations. The reliability of interaction evidence can vary due to its reliance on existing knowledge and curated data, potentially introducing biases. It mainly focuses on associations rather than physical interactions and has limited data from only a few model organisms, which may not always be applicable to other species. Extracting interaction data using text mining methods can be prone to errors due to naming conventions in literature. While the customization options are extensive, they may require users to have expertise to interpret combined

scores and additional evidence effectively. Enrichment analyses could be influenced by biases in the input data, and the database's coverage is more thorough for studied organisms, leaving gaps for less studied species. Continuous updates and enhancements are necessary to overcome these challenges and improve the reliability and usability of STRING[18].

Network analysis

In the present study, the Gephi software was utilized to the identification of critical node (betweenness and centrality > 200)[19]. Gephi is free software for graph and network analysis. It utilizes a 3D render engine to visualize large-scale networks in real time, expediting exploration. Its adaptable and multi-functional architecture provides new possibilities for managing complex datasets and producing meaningful visual results[20]. Also, Gephi faces several limitations, Especially when dealing with dynamic networks, the existing framework primarily focuses on static networks and requires plugins like DyCoNet for temporal data, which may not fully integrate with all functionalities. Using this software effectively requires considerable expertise in configuring and interpreting complex parameters and algorithms. The customization options, while extensive, are also intricate and time-consuming. Visualizing large or highly dynamic datasets can result in visual clutter and performance issues. Furthermore, Gephi's reliance on external plugins has the potential to cause compatibility and maintenance problems, and it may encounter scalability issues when handling very large datasets[21].

GTEx

GTEx database (https://gtexportal.org/home/) was utilized to find the tissue expression of the miRNAs. The database has created a data source and tissue bank to study the relationship between genetic types and gene expression In several human tissues and determine the gene expression level for each tissue separately[22]. The limitations of the GTEx project include significant variations in RNA quality extracted from different tissues, a limited number of samples for some tissues that can reduce the statistical power of Expression quantitative trait locus (eQTL) analysis, individual and tissue-specific differences that increase the complexity of interpreting results, constraints in splicing QTL (sQTL) analyses leaving many events undiscovered, reliance on data from deceased individuals which may cause potential differences in gene expression compared to living individuals, a low number of samples for specific tissues leading to potential over-generalizations, and the need for more data for comprehensive analyses. These limitations highlight the need for more advanced data and methods to understand better the genetic effects in different tissues and their impact on human diseases[23].

Enricher

In the present research, the Enrichr website (https://maayanlab.cloud/Enrichr/) was used to explore the molecular function and pathways of found genes. Enrichr integrates knowledge from many high-profile projects to provide biological data related to mammalian gene sets. The platform provides various methods to calculate the enrichment of gene sets, and the results are visualized in several interactive ways[24]. The limitations of Enrichr include the merging of human, mouse, and rat genes, which can lead to ambiguities, the lack of an ID conversion tool, which many users desire, and the inability to upload background lists for specific analyses. Additionally, Enrichr only supports parametric tests like Gene Set Enrichment Analysis (GSEA), Parametric Analysis of Gene Set Enrichment (PAGE), and Pathway Enrichment Analysis Algorithm (PAEA)., limiting its ability to perform more complex analyses. Also, the use of fuzzy gene sets, despite showing marginal improvements, still requires advanced computational expertise. Lastly, data quality in crowdsourced projects can vary and requires automatic and manual quality control[25].

Statistical analysis

After extracting gene data from various databases, a one-way ANOVA test was conducted to identify significant differences in gene expression between control and patient groups. In the network analysis using Gephi, various tests were performed, including the "Network Diameter Test," "Average Degree Test," "Degree Distribution Test," "Clustering Coefficient Test," "Degree Centrality Test," "Betweenness Centrality Test," and "Closeness Centrality Test"[26]. Significance was considered at a P value of less than 0.05 for all tests.

Ethical approval

This article does not contain any studies with human or animal subjects performed by any authors and does not require ethical approval and consent.

Results

Extraction of cervical cancer and HPV shared gene sets

In the present study, two gene sets were found, including 1818 related to cervical cancer and 430 related to HPV. Additionally, after sharing, 304 genes were extracted (Fig. 1) ($P < 0.05$).

Critical hob genes and network extraction

After finding 304 genes, using network analysis by the string website and removing them, ten proteins were detected, in order

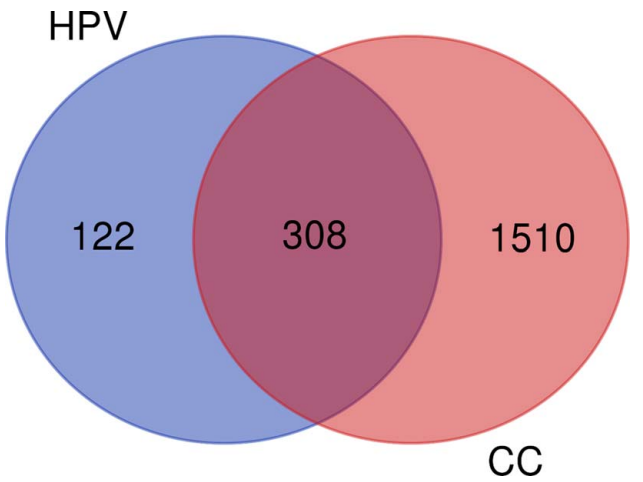

Figure 1. The shared genes among HPV and cervical cancer. HPV, human papillomavirus.

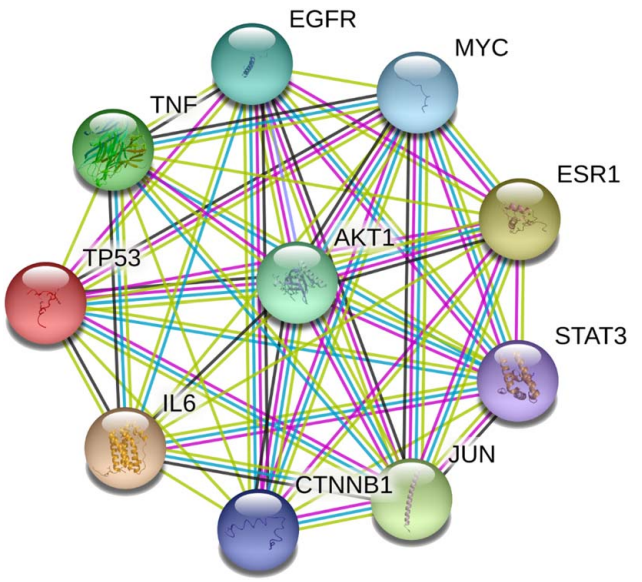

Figure 2. The important sub-network among human papillomavirus and cervical cancer.

from the highest betweenness to the lowest degree including TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN, respectively. These proteins have the highest level of interaction with each other's proteins, and after removing the sub-network, the major network suffered network failure (Fig. 2) (betweenness and centrality > 200).

miRNAs extraction

Shared miRNAs were extracted in the obtained gene set, including hsa-mir-146a, hsa-mir-27, hsa-mir-203, hsa-mir-126, hsa-mir-145, hsa-mir-944, and hsa-mir-93 (Table 1). Also, through text mining, the correlation between two disorders and miRNAs was validated. Correspondingly, the *P* value less than 0.05 was considered to include the validated relation (Table 2) ($P < 0.05$).

Tissue expression of the found miRNAs

By GTEx tool, the tissue expression of found miRNAs was investigated in 4 tissues endocervix, ectocervix, uterus, and vagina mode (Fig. 3). According to the findings, hsa-mir-145 has the highest expression in 4 tissues, and hsa-mir-93 is also expressed in the ectocervix.

Table 1

Shares miRNA among HPV and cervical cancer.

Origin genes	miRNA
MIR146A	hsa-mir-146a
MIR27A	hsa-mir-27
MIR203A	hsa-mir-203
MIR126	hsa-mir-126
MIR145	hsa-mir-145
MIR34A	hsa-mir-34
MIR944	hsa-mir-944
MIR93	hsa-mir-93

HPV, human papillomavirus.

Table 2

Correlation between HPV and cervical cancer.

	Significant correlation (0.05 >)	
miRNA	Cervical cancer	HPV
hsa-mir-146a	Upregulation	Upregulation
hsa-mir-27	Downregulation	Not validated
hsa-mir-203	Downregulation	Downregulation
hsa-mir-126	Downregulation	Downregulation
hsa-mir-145	Downregulation	Downregulation
hsa-mir-944	Upregulation	Upregulation
hsa-mir-93	Upregulation	Upregulation

HPV, human papillomavirus.

Discussion

In the present study, using bioinformatics data, ten proteins and seven miRNAs were extracted, which are shared between cervical cancer and HPV. These cases can be considered a gene expression and regulation network among HPV AND cervical cancer diseases.

Based on the current in-silico analysis, ten proteins, TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN, were identified as the most critical shared gene network among cervical cancer and HPV. Based on "Enrichr" data, the gene set is involved in Proteoglycans in cancer. This pathway is related to the extracellular matrix for cancer metastasis[27]. Also, the current network is involved in the incidence of endometrial and colorectal cancers and viral infections[28]. In addition, the present network is implicated in the up and downregulation of miRNAs involved in gene silencing[28]. In a study conducted in India, researchers revealed that TP53 and CTNNB1 show significant upregulation in patients with a history of HPV[29]. Additionally, evidence has indicated that HPV causes chromosomal instability in cervical cancer tumor lesions by affecting the TP53 gene[30]. Given the issue above, it can be deduced that the HPV virus has the potential to induce chromosomal instability or non-disjunction during mitosis. As evidenced by previous studies, this process may arise from the impact of high-risk HPV on the p53 protein, leading to increased DNA damage, notably due to micronuclei and double-strand breaks[31]. Likewise, Henrique *et al.*[32] showed that ESR1, JUN, TP53, and STAT3 genes are involved in HPV -induced cancer (neck or oropharyngeal). Li and colleagues demonstrated that the AKT1 gene promotes proliferation in cervical cancer cell lines by activating the estrogen signaling pathway, consequently heightening the risk of cancer metastasis[33,34]. Additionally, Ilahi *et al.*[35] demonstrated that the HPV virus, through the EGFR expression pathway, worsens the prognosis of cervical cancer. further, research conducted in 2006 showed that MYC activation caused by HPV DNA could be a crucial genetic event in cervical oncogenesis[36]. Other documents have also shown that TNF and IL6 genes produce proinflammatory cytokines, which create local immunity, suppress viral infection and prevent cervical cancer[37]. In addition, in the present study, one aspect of the gene network was examined, specifically focusing on the network density, which was analyzed

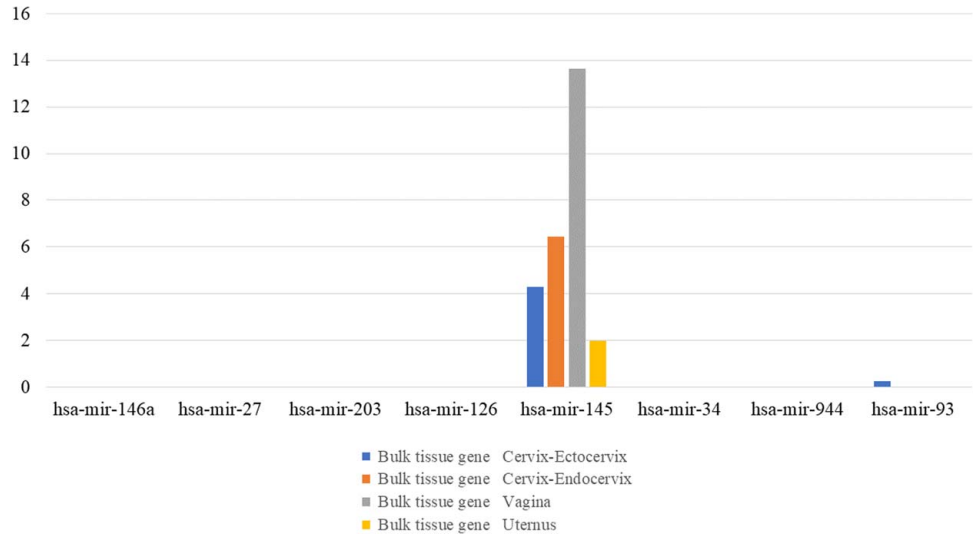

Figure 3. Tissue expression of extracted miRAN.

for the first time in this research. Based on previous studies, this theory can be considered as a platform for the development of gene therapy drugs. In this regard, by modifying this network (either by removing or enhancing nodes), significant changes in the treatment and management of viral diseases and their subsequent complications can be anticipated[19]. On the other hands; These identified proteins form a critical topological component of the shared networks between cervical cancer and HPV. It is important to emphasize that modifying the topology of biological networks is currently regarded as a therapeutic approach. For example, Emad Ramadan and colleagues have demonstrated that examining topological changes in biological networks during diseases, such as breast cancer, can provide an effective perspective for identifying the etiology of various cancers, including breast cancer[38]. Furthermore, genes involved in pathology are now analyzed as part of a network rather than in isolation[39]. Although the association of these proteins with cervical cancer or HPV infection is acknowledged in the existing literature, this study focuses on an intricate network that may serve as a therapeutic target for improving outcomes in cervical cancer caused by HPV infection. For instance, Du and colleagues have stated that altering and targeting intricate networks involved in the pathology of various cancers can lead to improved treatments[40]. However, according to the authors' knowledge, no evidence yet examines the current gene network in the incidence and prognosis of HPV-induced cervical cancer, and the network can be considered a therapeutic target for future studies.

According to bioinformatic data, seven miRNAs were found, including hsa-mir-146a, hsa-mir-27, hsa-mir-203, hsa-mir-126, hsa-mir-145, hsa-mir-944, and hsa-mir-93. Several studies showed hsa-mir-146a as a pleiotropic regulator of carcinogenesis[41]. Based on former evidence, HPV changes the miRNA expression patterns by utilizing its oncoproteins E5, E6 and E7. These viral proteins influence the expression of host genes, leading to irregularities in miRNAs. Specifically, E6 and E7 can increase or decrease miRNAs, impacting cellular functions like growth, cell death, and immune reactions. This disruption plays a role in advancing from HPV infection to cancer by targeting genes that suppress tumors and encouraging cancer-causing pathways[42]. On the other hands, In the context of cervical cancer, abnormal expression of miRNA plays a critical role in the onset, advancement, and progression of the disease. These miRNAs can function as oncogenes when their expression is increased or as tumor suppressors when their expression is decreased. Several mechanisms, including amplification, deletion, mutation at miRNA loci, as well as dysregulation of transcription factors and epigenetic signals, can lead to these alterations. The dysregulation of miRNAs can influence the expression of oncogenic or tumor suppressor proteins, thereby bringing about changes in the growth, invasion, and metastatic potential of cervical cancer cells. For example, miR-143 regulates apoptosis and inhibits cell growth by targeting Bcl-2, while miR-21 influences invasion and metastasis by targeting PDCD4. Additionally, changes in miRNA expression can impact cell cycle and apoptosis pathways through interactions with key signaling pathways such as Akt and mTOR[43]. Further, Wang and colleagues also revealed that miR-146a may be significantly overexpressed in HPV-positive history among cervical cancer patients[44]. hsa-miR-27 inhibits the progression of cervical adenocarcinoma through the reduction of TGFβ-R1[45], and this miRNA also plays a role in interfering with HPV virus replication[46]. Although former evidence has proved the relationship of hsa-miR-27 with cervical cancer[47] there is not sufficient evidence for the relationship between hsa-miR-27 and HPV-induced cervical cancer. Additionally, hsa-mir-203 is a crucial regulator of epidermal proliferation and differentiation, strongly downregulating in HPV-positive lesions[48]. Wilting *et al.*[49] in 2013 showed that hsa-mir-203 is methylated in cervical cancer lesions caused by HPV, which reduces the miRNA expression and worsens the prognosis of the disease. hsa-mir-126 is an endothelial-specific miRNA essential for maintaining vascular integrity during development, which is suppressed during cervical cancer occurrence[50]. Furthermore, Pulati *et al.*[51] revealed that hsa-mir-126 is downregulated during HPV infection. In addition, hsa-mir-944 is one of the miRNAs associated with HPV-induced cervical cancer, which causes the retreat of cervical cancer through the downregulation of the epidermal growth factor receptor (EGFR)

protein[52]. likewise, hsa-mir-93 also has significant upregulation during HPV-induced cervical cancer[53]. A cervical cancer according to tissue expression data, hsa-mir-145 has a significant expression in the cervix, uterus, and vagina tissues, which can be considered the most critical miRNA. In a 2019 study, Okoye *et al.*[54] found that hsa-mir-145 is overexpressed during cervical abnormalities. Moreover, Gunasekharan *et al.*[55] demonstrated that the HPV virus also causes genome amplification of human cells by modulating hsa-mir-145. hsa-mir-145 can be regarded as a major therapeutic target in cervical cancer malignancy, which requires more research to clarify. However, according to the authors, given that hsa-mir-145 has the strongest expression in the related tissues, it can be considered the most significant common factor between cervical cancer and HPV infection.

In the context of clinical application, the present findings hold potential applications across various areas. As growing evidence indicates, genes such as TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN play a crucial role in multiple cancer types[56]. Furthermore, HPV infection can alter the expression of these genes, which may ultimately lead to cervical cancer. In this regard, numerous studies have demonstrated promising outcomes in targeting these genes for cancer treatment. For example, evidence demonstrates that Nutlin can significantly inhibit tumor growth by targeting the p53-MDM2 complex[57]. Additionally, Bergmann *et al.*[58] have shown that targeting inflammatory cytokines like IL6 with compounds such as gp130 in a murine model can effectively prevent tumorigenesis by inducing DNA damage. Moreover, research by Ou and colleagues reveals that AKT1 can exacerbate tumorigenesis in cervical cancer. This finding is consistent with our results, indicating that AKT1 promotes carcinogenesis and that epigenetic factors, such as circular RNA targeting this protein, also play a significant role in tumor progression[59]. Another study showed that introducing a specific miRNA into the cell culture environment can lead to the expression of this gene, ultimately inducing apoptosis[60].

Moreover, estrogen is acknowledged as a carcinogen in many female cancers. In line with this, a study by Hernando *et al.*[61] has found that Selective Estrogen Receptor Degraders (SERDs) can significantly improve the prognosis of breast cancer. These findings collectively underscore the substantial potential for cancer treatment through gene therapy. However, it's important to note that some of the discussed interventions may not have undergone extensive preclinical and clinical testing, indicating that this area presents an attractive platform for future research.

Additionally, miRNAs hold significant potential for therapeutic, diagnostic, and prognostic applications. For instance, Wilczyński *et al.*[62] have noted that reduced tissue expression of miR-146a is linked to poorer prognosis in ovarian cancer patients, suggesting its viability as a biomarker. Similarly, Zhao *et al.*[63] have identified miR-27a as a promising therapeutic target for treating intrauterine adhesions (IUA), demonstrating that knockdown of miR-27a inhibits TGFβ1-induced epithelial-mesenchymal transition (EMT) and H2O2-induced oxidative stress in endometrial stromal cells (ESCs). further, Kawai *et al.*[64] have highlighted has-mir-126 in cervical mucus as a potential biomarker for cervical neoplasia, with its increased expression predicting cervical cancer occurrence with approximately 80% accuracy. Also, Okoye *et al.*[54] found that decreased serum levels of hsa-mir-145 correlate with cervical abnormalities, suggesting its potential as a biomarker. Park and colleagues reported that tissue expression of hsa-mir-944 is associated with a poorer prognosis in cervical cancer; however, they noted that the difficulty in accessing cervical tissue may limit its effectiveness as a biomarker[65]. Extensive in-vivo and in-vitro studies have demonstrated that miRNA-93 promotes tumor growth and survival. For example, Du *et al.*[66] showed that in NSCLC mouse xenografts, overexpression of miRNA-93 enhances tumor growth by downregulating specific targets. Additionally, Xu *et al.*[67], using qRT-PCR on EC tissues and cells, found that overexpression of miRNA-93-5p significantly enhances the viability and migration of EC cells, which is mediated through the modulation of the interferon-alpha and beta receptor subunit 1 (IFNAR1). In summary, these findings suggest that the identified miRNAs have substantial potential as biomarkers for diagnosis and prognosis and therapeutic targets in cancer treatment.

Furthermore, with the aging process, many cellular markers may undergo changes in the identified profile[68]. For instance, it has been shown that TP53 can exhibit dual behavior with advancing age, potentially leading to cancer development[69]. Additionally, the expression profiles of many genes change with age. According to findings by Zabihi *et al.*[70], these changes can influence the survival and death of various diseases by affecting immune checkpoints. Moreover, Smith and colleagues have demonstrated that aging can alter miRNA profiles, which may impact the progression or improvement of numerous pathologies[71]. Lastly, it is important to highlight that the interplay between aging and various pathologies represents a significant avenue for future research endeavors.

Limitations

Given that the present study is a bioinformatics investigation, it may encounter significant challenges and limitations. First issue: Due to resource constraints, this study lacks experimental validation. Conducting this process would enhance the value of the obtained data. Second issue: Since this research examines the pathway from protein to microRNA, some important cases may have been overlooked. Third issue: Additionally, considering the relatively novel status of non-coding RNAs in clinical and research processes and the lack of sufficient empirical observations, the authors were unable to adequately establish and demonstrate the strength of the relationship between the identified microRNAs and HPV-related complications, particularly cervical cancer. Additionally, this research utilized bioinformatics methods, which come with their own set of limitations. In this regard, one of the main challenges in bioinformatics is the inconsistency in data analysis methods, annotation, and connectivity, which pose significant obstacles to its integration.

Implications for clinical practice

The study's findings suggest several clinical applications for managing HPV-induced cervical cancer. Key hub proteins and critical miRNAs identified in the research can serve as valuable biomarkers for early diagnosis and personalized treatment plans. Targeting upregulated miRNAs (such as hsa-mir-145) or upregulating downregulated miRNAs (like hsa-mir-27, hsa-mir-203, and hsa-mir-126) presents innovative gene therapy strategies. Integrating these molecular insights into existing diagnostic and therapeutic protocols could enhance patient care, improve treatment outcomes, and better manage HPV-related complications.

Recommendations for future studies

In order to strengthen the reliability of our data, future studies should include experimental validation to support the computational findings. It is important to conduct comprehensive pathway analysis to identify any overlooked pathways. Additionally, extensive empirical studies are necessary to establish the strength of the relationship between the identified miRNAs and HPV-related complications. Integrating multi-omics data will provide a more complete understanding of molecular interactions. Furthermore, studying the tissue-specific expression of key miRNAs will help to clarify their roles in the development of cervical cancer. Also, examining the molecular mechanisms through which the identified proteins and miRNAs contribute to carcinogenesis. It is also recommended to carry out longitudinal and large-scale studies with diverse populations to validate findings and ensure their generalizability.

Conclusion

According to the data and network analysis, ten critical hub proteins (TP53, MYC, AKT1, TNF, IL6, EGFR, STAT3, CTNNB1, ESR1, and JUN) may be essential for maintaining the network integrity needed to initiate cervical cancer after HPV infection. Furthermore, we identified epigenetic factors that regulate this network and can influence the onset of the pathology. These factors include key miRNAs (hsa-mir-146a, hsa-mir-27, hsa-mir-203, hsa-mir-126, hsa-mir-145, hsa-mir-944, and hsa-mir-93), which have been confirmed to have significant correlations (P value <0.05). Additionally, tissue expression analysis revealed that hsa-mir-145 is most highly expressed in the endocervix, ectocervix, uterus, and vagina, while hsa-mir-93 is notably expressed in the ectocervix. These findings suggest the potential role of gene networks and miRNAs in the pathogenesis of HPV-induced cervical cancer and indicate possible therapeutic targets. However, these preliminary results require further functional validation through experimental follow-up to confirm their therapeutic relevance and potential clinical applications.

Ethical approval

This article does not contain any studies with human or animal subjects performed by any authors and does not require ethical approval and consent.

Consent

Not applicable.

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Author contribution

Study concept and design by all authors; Data acquisition by all authors; Data interpretation by all authors; drafting the manuscript by all authors; Revision of the manuscript by all authors; the final version of the manuscript is approved by all authors.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Research registration unique identifying number (UIN)

We could not register our manuscript in the Research Registry UIN: www.researchregistry.com due to internet access restrictions and international sanctions. we live in Iran. We hardly even meet the basic needs of our daily life. We do not receive any funding for our research and we cannot pay for our research. Please excuse us from registering this manuscript in the Research Registry UIN: www.researchregistry.com.

Guarantor

Samad Karkhah.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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