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ORIGINAL ARTICLE

Exploring the Correlation between MicroRNA-128 and MicroRNA-212 Expression and Cytokine Profiles in HPV-Induced Wart Patients: Implications for Immune Regulation and Targeted Therapeutic Approaches

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Key words:
MicroRNA; HPV-induced Warts; Cytokines biomarkers; Immune Modulation; Therapeutic Targets; Correlation

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ABSTRACT

Background *Human papillomavirus infections have previously been linked to causing warts, and microRNAs (miRNAs) have been reported to play modes to modulate the immune response to these type infections.* ***objective:*** *This study examined the relationship between miR- 128/miR-212 expression and cytokine levels in HPV-related warts, exploring their role in immune responses and potential as therapeutic targets for HPV treatment.* ***Methodology:*** *miR-128 and miR-212 expression levels were measured by qRT-PCR in tissue samples, while levels of IFN-γ, TNF-α, and IL-10 cytokines were measured by ELISA in blood samples from 150 patients with HPV-induced warts.* ***Results:*** *Expression of miR-128 and miR-212 significantly correlated with cytokine profiles in HPV-induced warts. MiR-128 showed a strong positive correlation with IFN-γ, suggesting involvement in antiviral immunity. Weaker positive correlations existed between miR-128 and both TNF-α and IL-10. In contrast, miR-212 moderately negatively correlated with IFN-γ and had a slight positive correlation with TNF-α and IL-10. These results suggest an immunomodulatory role for miR-128 and indicate that the influence of miR-212 is less powerful.* ***Conclusions:*** *The correlation between expression of miR-128 and high IFN-γ revealed that miR-128 is a potential therapeutic target when using IFN-γ injection to treat HPV-induced warts and encourage more investigation of miR-128/miR-212 in the immune response of HPV infection and novel therapeutic strategies against HPV-induced warts.*

INTRODUCTION

Human papillomavirus (HPV) infection, a global health concern, is implicated in the development of warts and various cancers, most notably cervical cancer, impacting millions and imposing a substantial disease burden[1]. Host immunity plays a crucial role in HPV infection control; however, the underlying mechanisms remain incompletely elucidated. Advancing knowledge of HPV, immune responses, and microRNA (miRNA) interactions is essential for improved preventative and therapeutic strategies[1,2]. Prior investigations have explored the involvement of various miRNAs in HPV infection [2,3]. This study aimed to clarify the roles of miR-128 and miR-212 in modulating HPV-associated immune responses. Elucidating these miRNAs' functions may contribute to the development of enhanced HPV prevention and treatment modalities. When examining the modulatory effects of miR-128 and miR-212 on immunity against human papillomavirus (HPV), all three cytokines—IFN-γ, TNF-α, and IL-10—will be discussed during this investigation. It also discusses their possible value as biomarkers and therapeutic targets for HPV-related diseases. The precise ways these processes occur and the clinical ramifications of these processes in HPV-induced warts are still not completely understood [4].

While miRNA expression is significant in HPV-associated warts, some studies note a correlation between individual miRNA and cytokine levels, and high variation in expression exists which ultimately limits the clinical significance of miRNA 1,5. The interactions between HPV type, host genetics, and corresponding cytokine profiles complicate interpretation and hinder the design of efficient targeted therapy [5,6] . Other reports suggest that enhanced immune responses to SARS-CoV-2, whether through natural infection or vaccination, may correlate with greater protection against HPV. [7,8,9,10]. The purpose of this study is to investigate the correlation between the expression of microRNA-128 (miR-128) and microRNA-212 (miR-212) and cytokine profiles in patients with HPV-induced warts, aiming to explore their roles in immune regulation and potential as therapeutic targets for HPV-related diseases.

METHODOLOGY

The objective of this cross-sectional study was to evaluate the association of miR-128 and miR-212 expression with IFN-γ, TNF-α, and IL-10 cytokines in patients with HPV-induced warts.

The study recruited 150 patients (≥18 years) with clinically diagnosed HPV-induced warts (skin, plantar, genital) from October 2023–March 2024 visiting Dermatology Departments in Al-Ramadi and Fallujah, whereas patients with autoimmune diseases, immunosuppression, pregnancy, breastfeeding, prior wart treatment, or other confounding conditions were excluded ensuring a homogenous study population for accurate assessment of cytokine levels and microRNA expression. Informed consent was obtained from all participants. The sample size was calculated based on a power of 80% and a significance level of 0.05 using G Power 3.1 software, resulting in 150 warts related HPV.

Ethics Approval Committee

This study was conducted following the Declaration of Helsinki, and was approved by the Committee on Medical Ethics of the University of Anbar (Permission number 155, March 12 2023).

Serological Assessment

Fasting serum samples were collected in 5 ml EDTA-anticoagulated venous blood and centrifuged for 10 min at 1500 g to obtain serum which were stored at -80°C. Serum concentrations of IFN-γ, TNF-α and IL-10 were performed in duplicate using specific enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech Co., Korea) by reading the absorbance at 450 nm in a Bio-Rad microplate reader.

Molecular Analysis

A- Sample Collection and Processing:

Four-millimeter punch biopsies were taken from wart lesions under local anesthesia using sterile techniques. Samples were immediately frozen in liquid nitrogen and stored at -80°C for subsequent molecular analysis [11].

B- miRNA Extraction:

Total RNA, including microRNAs miR-128 and miR-212, was extracted from tissue samples using TRIzol reagent (Bioneer, Korea), phase separation is achieved with acid-phenol–chloroform to isolate the aqueous phase containing miRNAs. Ethanol is added to precipitate RNA, which is then filtered, washed, and eluted using nuclease-free water. RNA quality and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

C- Quantification of DNA

For quantification, miRNAs are reverse transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Bioneer, Korea) to generate cDNA. miR-128 and miR-212 expression levels were quantified using TaqMan MicroRNA assays (Bioneer, Korea) in a qRT-PCR reaction on a 7500 Fast Real-Time PCR System 11. Relative expression levels were normalized to U6 snRNA using the 2ΔΔCt method to determine relative expression levels [11]. (Table 1)

Table 1: miR212 and miR128 gene primers were used in this research

Gene	The sequence of primer	Size of fragment in base pairs (bp)
miR212	F AACACGTGACCTTGGCTCTAGA	22
miR128	F AAGAATCGGGGCCGTAGCA	19
Universal reverse primer	CAGTGCAGGGTCCGAGGT	18
U6F	GTGCTCGCTTCGGCAGCA	18
U6R	CAAAATATGGAACGCTTC	18

D- Methodological Considerations:

Tissue was homogenized for total RNA extraction with TRIzol reagent 11. and miR-128 and miR-212 expression were quantified by TaqMan assays and normalized to U6 snRNA. That approach worked out quite well.

Statistical analysis:

Statistical analysis was carried out using SPSS (v. 27.0) and GraphPad Prism. MicroRNA and cytokine levels (continuous variables) were expressed as mean ± SD, and their normality was examined using Shapiro-Wilk or Kolmogorov-Smirnov tests. The correlation between microRNA and cytokine levels was assessed with Pearson correlation analysis ($p < 0.05$). A p-value < 0.05 was considered statistically significant.

RESULTS

In our work we found a strong positive correlation between miR-128 expression and IFN-γ levels ($r(98) = 0.86$, $p < 0.0001$) (**Figure 1**), confirming an association between miR-128 expression and IFN-γ levels. The correlation coefficient ($r^2 = 0.74$) indicates that the miR-128 expression can explain about 74% of the variance in IFN-γ concentrations. These results strongly support a positive correlation between miR-128 and IFN-γ in HPV-induced warts providing additional insights towards our hypothesis. The regression investigation determines) additional determines this association, with a 95% confidence interval of 0.8115 to 0.8966. (**Figure 1**)

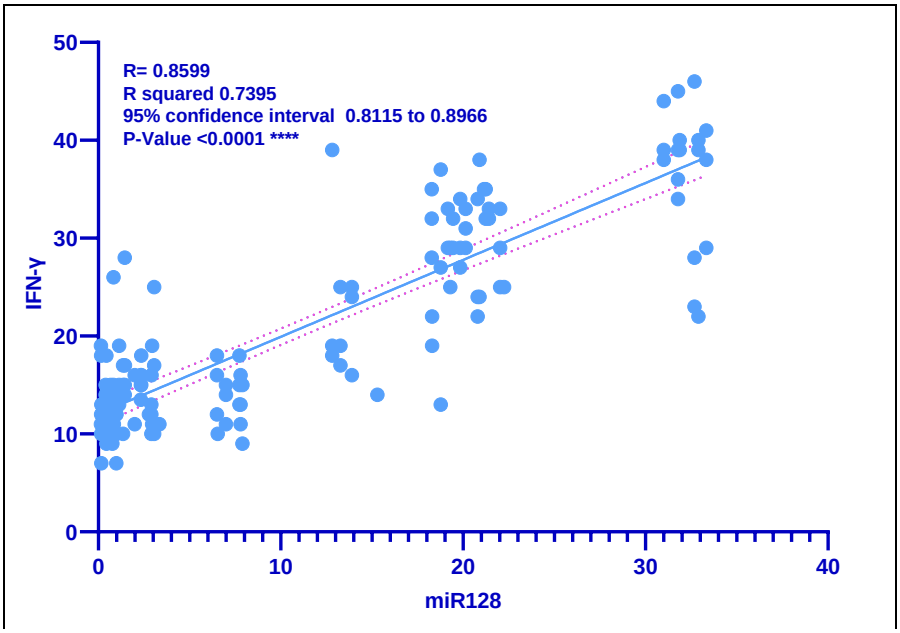

Fig. 1: Correlation of microRNA 128 with interferon gamma in patients with warts due to HPV infections

A statistically significant moderate negative correlation (r (98) = -0.52, p < 0.0001) (inverse relationship) was observed between miR-128 and TNF-α, where the higher level of miR-128 correlated with lower level of TNF-α. The same argument can be applied to TNF-α levels, with miR-128 expression accounting for approximately 27% of the variance in TNF-α levels (r^2 = 0.27). Thus, the present findings provide relevant evidence on the strong inverse association between miR-128 and TNF-α levels in HPV-induced warts. (**Figure 2**)

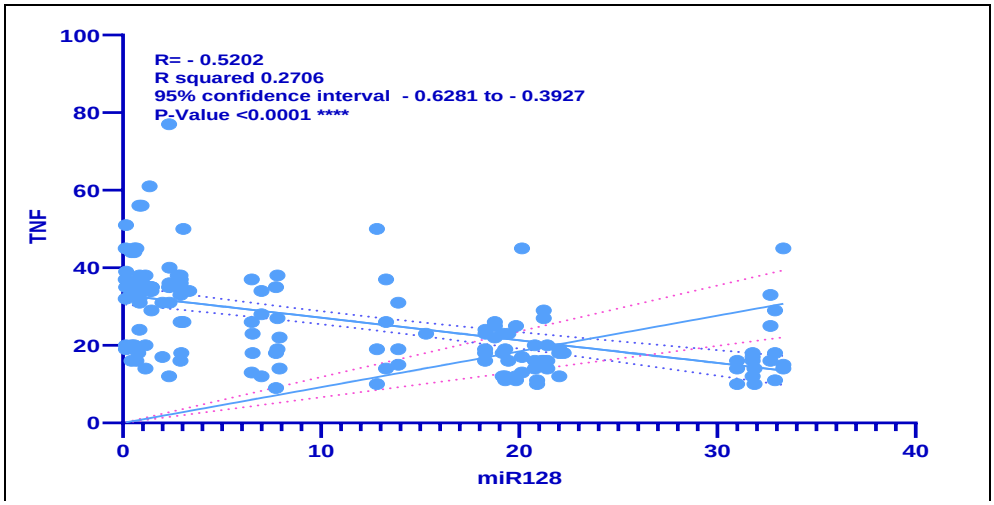

Fig. 2: Correlation of microRNA 128 with tumor necrotizing factor (TNF) in patients with warts due to HPV infections

The increased expression of miR-128 was weakly negatively related with the levels of IL-10 with a significant p value (r (98) = -0.31, p < 0.0001;. Further miR-128 expression was related with lower IL-10 levels but the effect size was modest. The coefficient of determination (r^2 = 0.10) indicates that miR-128 expression accounts for only approximately 10% of the variance in IL-10 levels, suggesting limited descriptive power in this environment. **(Figure3**)

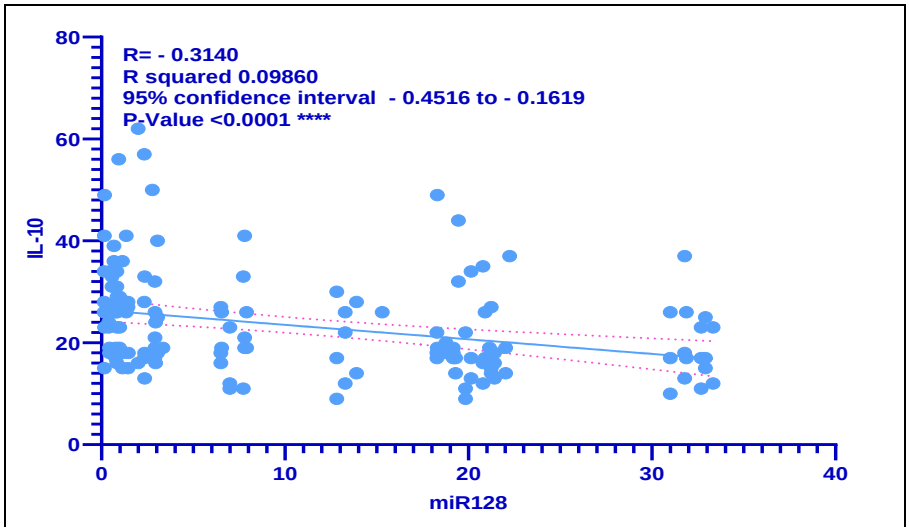

Fig. 3: Correlation of microRNA 128 with interleukin 10 (IL-10) in patients with warts due to HPV infections

This association was statistically important and moderate (r (98) = - 0.51, p < 0.0001) (Figure 4). A greater expression of miR-212 associated with minor levels of IFN-γ. The value of r^2 = 0.26 shows that miR-212 explains around 26% of the variance in IFN-γ (e), suggesting that the significant link between miR-212 and IFN-γ might be the consequence of further factors. These results show a strong negative correlation of miR-12 vs. IFN-γ in HPV−induced warts. (**Figure-4**)

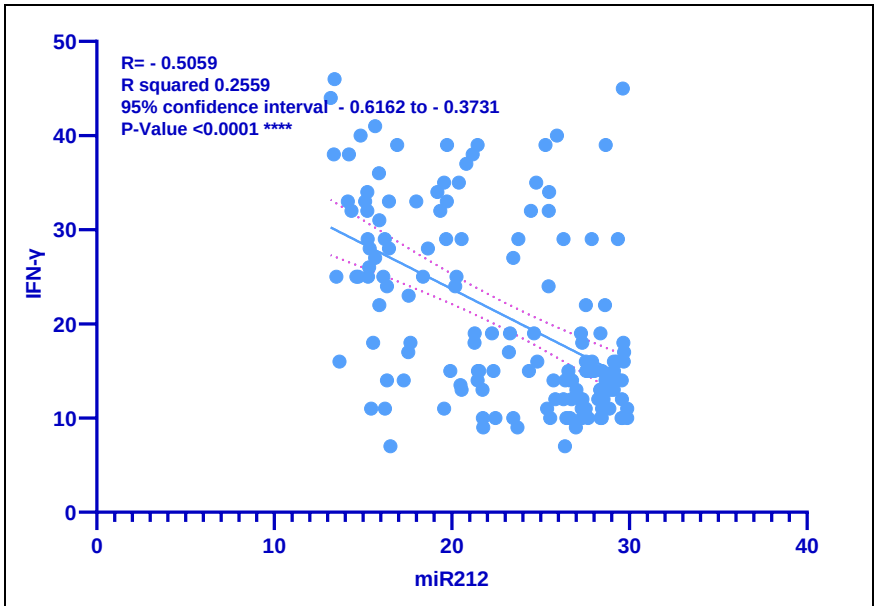

Fig. 4: Correlation of microRNA 212 with interferon gamma in patients with warts due to HPV infections

The relationship between miR-212 expression and TNF-α as well as IFN-γ levels was analyzed in HPV-positive wart patients (Figure 5). A weak positive correlation between miR-212 and TNF-α was observed, yet statistically significant (p = 0.0078, r = 0.2166, r^2 = 0.0384), which seems to point to a limited contribution of miR-212 to the variance of TNF-α. In contrast, a statistically significant moderate negative correlation was seen between miR-212 and IFN-γ (r^2 = 0.2559, p <0.01), which may emphasize a more considerable though still partial effect of this miRNA on IFN-γ levels. These findings suggest that miR-212 may act as a factor in the complex relationship between microRNA and cytokine networks in HPV-induced warts**. (Figure 5)**

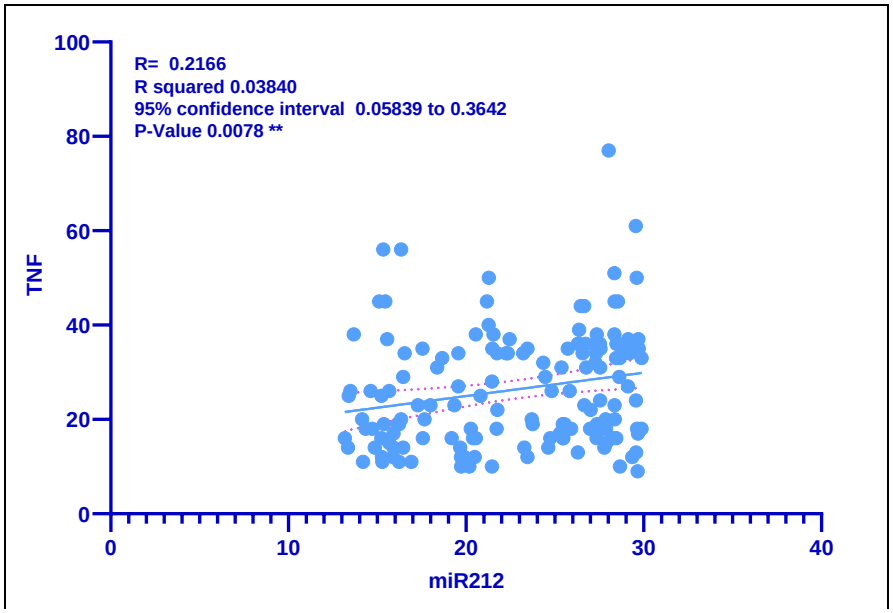

Fig. 5: Correlation of microRNA 212 with tumor necrotizing factor (TNF) in patients with warts due to HPV infections

MiR-212 expression weakly positively correlated with IL-10 levels in patients with HPV-induced warts (r (98) = 0.21, p = 0.0083) (Figure 6). Thus, high expression of miR-212 associated slightly elevated levels of IL-10. MiR-212, however, explained only about 4% ($r^2 = 0.04$) of variability in IL-10 levels. Such correlation is statistically significant but of rather limited practical value.(**Figure 6**)

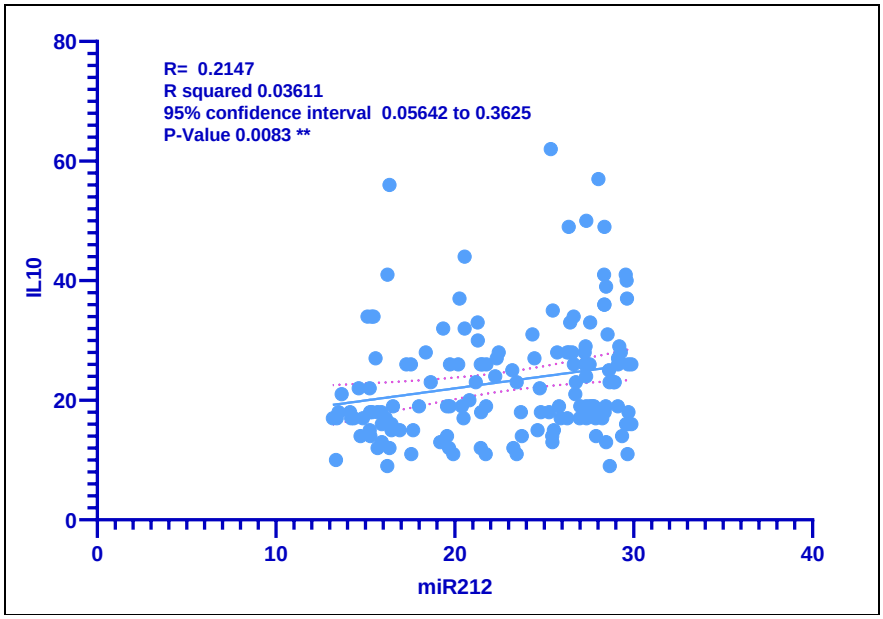

Fig. 6: Correlation of microRNA 212 with Interleukin -10 (IL-10) in patients with warts due to HPV infections

DISCUSSION

The study reveals miR-128 and miR-212 as key immune modulators in HPV infection. miR-128 positively correlates with IFN-γ, enhancing antiviral immunity, while negatively with TNF-α and IL-10. miR-212 negatively correlates with IFN-γ, suggesting immune evasion. Both show potential as biomarkers and therapeutic targets for HPV-related diseases. The results align well with previous studies that have established the regulatory roles of microRNAs in viral infections and immune responses. For instance, the strong positive correlation between miR-128 and IFN γ is consistent

with earlier reports on the immunomodulatory functions of miR-128 during viral infections[1,3,12,13,14,15]. This supports the notion that miR-128 enhances antiviral immunity, potentially making it a promising therapeutic target for HPV-related diseases[15-17]. Similarly, the negative correlation observed between miR-212 and IFN γ corroborates prior findings that some microRNAs suppress cytokine production to facilitate viral immune evasion [18-20]. However, the weak associations of miR-128 and miR-212 with TNF-α and IL-10 contrast with some studies, highlighting the context-dependent nature of microRNA functions [4,14,15,21,22]. These discrepancies may arise from differences in study designs, sample sizes, or biological contexts, emphasizing the need for further research to clarify these relationships.

The study's findings align with prior research on miR-128's role in antiviral immunity but contrast with some reports showing stronger miR-128/IL-10 associations, possibly due to differing experimental conditions or HPV genotypes [15,16]. Similarly, the weak miR-212/TNF-α correlation diverges from studies reporting significant miRNA-cytokine interactions in other viral infections 18,19. These discrepancies highlight the context-dependent nature of miRNA functions. Additionally, while this study focused on HPV-induced warts, other studies emphasize miRNAs' roles in HPV-related cancers, suggesting broader applications [6,14]. The lack of genotype-specific analysis further limits comparisons, as HPV types may differentially modulate miRNA expression and immune responses [1,23,24]. Future research should address these gaps for a comprehensive understanding.

However, discrepancies exist in the literature regarding the specific roles of miR-128 and miR-212. For example, while this study found only a weak association between miR-128 and IL-10, other studies have suggested more pronounced effects of miR-128 on inflammatory cytokines [5,15,25,26]. Such contradictions may stem from differences in experimental conditions or biological contexts, emphasizing the need for further research to clarify these inconsistencies. Internationally, studies have highlighted the dual roles of microRNAs in inflammation—acting either as suppressors or stimulators of cytokine production [3,13,16,27]. This complexity is evident in the current findings, where miR-128's moderate negative correlation with TNF-α contrasts with its strong positive correlation with IFN-γ. The context-dependent behavior of microRNAs underscores the importance of understanding their mechanisms within specific pathological settings.

The study highlights miR-128 and miR-212 as potential therapeutic targets for HPV-related diseases. miR-128 enhances antiviral immunity via IFN-γ and modulates inflammation via TNF-α and IL-10, while miR-212 may reverse immune evasion by inhibiting IFN-γ. Both could serve as biomarkers and inform microRNA-based therapies for HPV-induced lesions like cervical cancer. The study's limitations include not accounting for HPV genotype variations, a small sample size, and unclear miRNA-cytokine mechanisms, restricting result generalizability and requiring further validation and research.

Conclusion: The study highlights miR-128 and miR-212 as regulators of HPV immune responses, with miR-128 enhancing antiviral immunity and miR-212 potentially aiding immune evasion. Further research is needed to clarify mechanisms, validate findings, and explore HPV genotype impacts.

CONCLUSION

The study identified an association between miR-128 and miR-212 and cytokine profiles in HPV warts; because miR-128 is positively correlated with IFN-γ, it proposes itself as a candidate for a possible immunotherapeutic target to boost antiviral immunity, though additional work is warranted toward validating this point and elucidating the therapeutic implications of miR-128 modulation and the functions of both microRNAs in HPV pathogenesis. Novel miRNA-based interventions targeting key miRNAs involved in the HPV immune response can help enhance viral clearance and reduce disease severity. Although this model and findings should be studied more to be applicable in wider context, this study contributes to the understanding of the interaction between miRNAs and the immune system during HPV infection.

Declarations of 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."

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