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Research article

Integrative analysis of gene expression and DNA methylation to identify biomarkers of non-genital warts induced by low-risk human papillomaviruses infection

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ABSTRACT

Background: Human papillomaviruses have been shown to dysregulate the gene expression and DNA methylation profiles of their host cells over the course of infection. However, there is a lack of information on the impact of low-risk HPV infection and wart formation on host cell's expression and methylation patterns. Therefore, the objective of this study is to analyse the genome and methylome of common warts using an integrative approach.

Methods: In the present study, gene expression (GSE136347) and methylation (GSE213888) datasets of common warts were obtained from the GEO database. Identification of the differentially expressed and differentially methylated genes was carried out using the RnBeads R package and the edgeR Bioconductor package. Next, functional annotation of the identified genes was obtained using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). Network construction and analyses of the gene-gene, protein-protein, and signaling interactions of the differentially expressed and differentially methylated genes was performed using the GeneMANIA web interface, the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, and the Signaling Network Open Resource 2.0 (SIGNOR 2.0), respectively. Lastly, significant hub genes were identified using the Cytoscape application CytoHubba.

Results: A total of 276 genes were identified as differentially expressed and differentially methylated in common warts, with 52% being upregulated and hypermethylated. Functional enrichment analysis identified extracellular components as the most enriched annotations, while network analyses identified *ELN, ITGB1, TIMP1, MMP2, LGALS3, COL1A1* and *ANPEP* as significant hub genes.

Conclusions: To the best knowledge of the authors, this is the first integrative study to be carried out on non-genital warts induced by low-risk HPV types. Future studies are required to re-validate the findings in larger populations using alternative approaches.

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1. Introduction

Human papillomaviruses (HPV) are a family of epitheliotropic DNA viruses that infect the mitotically active basal keratinocyte layer in the cutaneous and mucosal epithelia [1]. More than 200 types of HPV have been identified and they are grouped into three main categories: cutaneous, mucocutaneous, and those types associated with epidermodysplasia verruciformis, a rare autosomal recessive disorder [2,3]. Several types of HPV are classified as high-risk due to their carcinogenic potential, but the majority of HPV types are low risk and cause benign hyper-proliferative lesions known as warts [4]. In immunocompetent individuals, warts are self-limiting and spontaneously cleared as a result of cell-mediated immunity [5].

HPV-related diseases have been the subject of a wide range of omics studies in recent years, generating a huge number of datasets. However, such omics data is highly dimensional and requires complex analysis in order to extract relevant biological insights [6]. To overcome this challenge, integrative biological approaches - combining more than one type of omics data - have been introduced for malignancies associated with high-risk HPV infection, including cervical cancer, head and neck cancer, and oropharyngeal cancer [7–11].

In contrast, research on low-risk HPV infection has not been prioritized due to its typically benign effects, and information about the impact of HPV-driven wart formation on host gene expression and methylation remains sparse [4]. Therefore, this study aims to identify and analyse genes which are both differentially expressed and differentially methylated (DEDM) in warts. To the best of our knowledge, this study is the first to apply an integrative analysis of gene expression and methylation in non-genital warts caused by low-risk HPV infection.

Table 1
List of the top DEDM genes in terms of expression and methylation status.

Gene	logFC	FDR	mean.mean.diff (W-NS)	Combined rank score	Expression	Methylation
Top upregulated DEDM genes						
CYB561A3	1.840251	1.73×10^{-11}	0.047118	4541	Up	Hyper
FCGRT	2.979764	2.89×10^{-11}	0.041616	4214	Up	Hyper
TNFSF12	2.25828	8.18×10^{-11}	0.038217	4873	Up	Hyper
LRRN4CL	3.924778	2.53×10^{-10}	0.062005	2158	Up	Hyper
RELL1	1.59098	4.76×10^{-10}	0.041974	7093	Up	Hyper
IGFBP4	2.51739	6.88×10^{-10}	0.03439	5789	Up	Hyper
SHISA4	2.395266	1.93×10^{-09}	0.033263	6579	Up	Hyper
SOX10	2.762969	1.97×10^{-09}	0.062117	5707	Up	Hyper
C9orf152	5.112678	2.05×10^{-09}	−0.06451	1908	Up	Hypo
PAMR1	3.767645	2.84×10^{-09}	0.044544	3774	Up	Hyper
Top downregulated DEDM genes						
SAMD9	−4.3315	1.02×10^{-11}	−0.10514	1620	Down	Hypo
OASL	−5.41896	1.40×10^{-11}	−0.03514	5566	Down	Hypo
FABP5	−2.71433	1.41×10^{-11}	−0.06302	4249	Down	Hypo
FUT3	−3.28519	1.65×10^{-11}	−0.03794	6010	Down	Hypo
KRT6A	−6.81118	1.82×10^{-10}	−0.17458	687	Down	Hypo
GJB6	−3.05702	3.07×10^{-10}	−0.0811	1150	Down	Hypo
GJB2	−3.40401	3.11×10^{-10}	−0.27221	54	Down	Hypo
KRT6C	−9.14735	3.95×10^{-10}	−0.04832	6118	Down	Hypo
IL36G	−3.68497	4.56×10^{-10}	−0.06271	2026	Down	Hypo
S100A16	−1.3326	5.03×10^{-10}	−0.06332	1985	Down	Hypo
Top hypermethylated DEDM genes						
FABP7	4.427163	1.60×10^{-05}	0.197528	134	Up	Hyper
PHYHD1	2.562795	3.94×10^{-08}	0.183384	356	Up	Hyper
COX7A1	1.549866	1.67×10^{-05}	0.220857	456	Up	Hyper
STAT5A	1.360049	1.14×10^{-05}	0.118592	458	Up	Hyper
MFAP4	4.089278	1.67×10^{-06}	0.143125	593	Up	Hyper
IL11RA	2.775918	1.59×10^{-07}	0.127114	604	Up	Hyper
BHLHE41	1.76519	1.72×10^{-06}	0.116557	650	Up	Hyper
MFAP2	1.806981	2.77×10^{-06}	0.116759	829	Up	Hyper
TFAP2B	1.444659	2.58×10^{-06}	0.117012	837	Up	Hyper
RARRES3	2.305639	1.20×10^{-03}	0.089248	923	Up	Hyper
Top hypomethylated DEDM genes						
AREG	−3.39287	1.38×10^{-09}	−0.40848	17	Down	Hypo
GJB2	−3.40401	3.11×10^{-10}	−0.27221	54	Down	Hypo
S100A8	−8.4116	1.07×10^{-08}	−0.16681	164	Down	Hypo
ZBED2	−1.84179	1.10×10^{-05}	−0.17879	169	Down	Hypo
SPINK6	−9.92748	3.67×10^{-09}	−0.27988	265	Down	Hypo
CALML3	−1.71347	8.83×10^{-04}	−0.13474	305	Down	Hypo
S100A7	−7.53527	2.76×10^{-08}	−0.12511	414	Down	Hypo
S100A9	−8.6431	8.98×10^{-09}	−0.15165	551	Down	Hypo
LGALS9C	−3.30056	7.32×10^{-07}	−0.10988	554	Down	Hypo
SFN	−1.20378	4.04×10^{-05}	−0.10243	675	Down	Hypo

2. Results

2.1. Differential methylation and expression analysis

Upon matching the DM (from the RnBeads analysis) and DE (from the edgeR analysis) genes, 276 genes were identified as both differentially expressed and differentially methylated (DEDM) in common warts (Table 1). The most upregulated DEDM genes were *CYB561A3*, *FCGRT*, and *TNFSF12,* while the most downregulated DEDM genes were the *SAMD9*, *OASL*, and *FABP5*. Likewise, *FABP7*, *PHYHD1*, and *COX7A1* were the most hypermethylated DEDM genes, and *AREG*, *GJB2*, and *S100A8* were the most hypomethylated DEDM genes.

Fig. 1A illustrates that the majority of DEDM genes (52%) were both upregulated and hypermethylated, while Fig. 1B shows that chromosome 1 has the highest number of DEDM genes (n = 40), which is unsurprising as chromosome 1 is the largest human chromosome.

2.2. Gene annotation

After identifying the DEDM genes in common warts, we used the Database for Annotation, Visualization, and Integrated Discovery (DAVID) web server to functionally annotate and thereby derive the biological meaning behind the DEDM genes. Table 2 lists the top most enriched annotations among the 276 DEDM genes in common warts.

Most prominently, the most enriched disease was palmoplantar keratoderma ($p = 7.49 \times 10^{-09}$), and the proteins secreted in the surroundings were the most enriched functional annotation ($p = 1.75 \times 10^{-08}$). Regarding gene ontology, the extracellular region ($p = 4.41 \times 10^{-10}$) and the extracellular space ($p = 5.57 \times 10^{-10}$) were the most enriched GO terms. Similarly, the chemokine (C–C motif) receptor 1 (CCR1) ($p = 1.79 \times 10^{-09}$) and chromosome 18 open reading frame 21 (C18ORF21) ($p = 6.79 \times 10^{-09}$) proteins were the most enriched interactions. Lastly, the formation of the cornified envelope ($p = 2.93 \times 10^{-10}$) was the most enriched pathway, and the S-100/ICaBP type calcium binding domain ($p = 4.64 \times 10^{-07}$) was the most enriched protein domain.

Fig. 2 provides a further analysis of the most enriched GO terms in the DEDM genes, confirming the dominance of extracellular

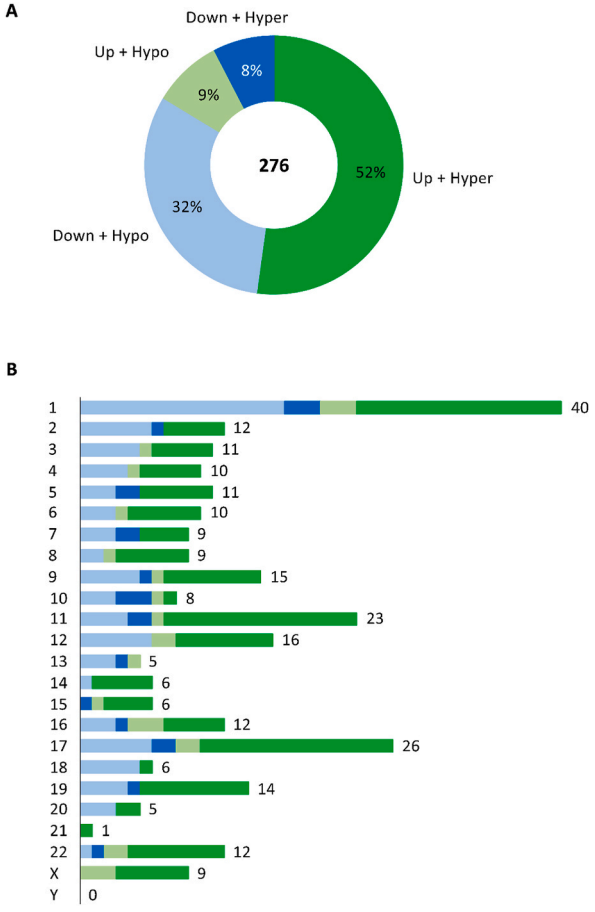

Fig. 1. Expression and methylation status (a) and chromosomal location of DEDM genes (b) in common warts.

Table 2
Functional annotation of the 276 DEDM genes in warts.

Category	Keyword	Term	Count	FDR
Diseases				
UP_KW_DISEASE	KW-1007	Palmoplantar keratoderma	11	7.49×10^{-09}
UP_KW_DISEASE	KW-0038	Ectodermal dysplasia	7	3.34×10^{-03}
DISGENET	C4551675	Keratoderma, Palmoplantar	6	4.93×10^{-03}
DISGENET	C0023893	Liver Cirrhosis, Experimental	31	6.10×10^{-03}
DISGENET	C0011616	Contact Dermatitis	8	4.67×10^{-02}
Functional Annotations				
UP_KW_CELLULAR_COMPONENT	KW-0964	Secreted	66	1.75×10^{-08}
UP_SEQ_FEATURE	–	CARBOHYD:N-linked (GlcNAc …) asparagine	99	2.06×10^{-05}
UP_KW_CELLULAR_COMPONENT	KW-0403	Intermediate filament	10	3.53×10^{-05}
UP_KW_PTM	KW-0325	Glycoprotein	107	3.53×10^{-05}
UP_KW_PTM	KW-1015	Disulfide bond	90	1.80×10^{-02}
Gene Ontology				
GOTERM_CC_DIRECT	GO:0005576	extracellular region	69	4.41×10^{-10}
GOTERM_CC_DIRECT	GO:0005615	extracellular space	64	5.57×10^{-10}
GOTERM_CC_DIRECT	GO:0001533	cornified envelope	10	5.35×10^{-07}
GOTERM_CC_DIRECT	GO:0070062	extracellular exosome	56	1.63×10^{-04}
GOTERM_BP_DIRECT	GO:0031424	keratinization	10	1.22×10^{-03}
Interactions				
BIOGRID_INTERACTION	1230	CCR1	28	1.79×10^{-09}
BIOGRID_INTERACTION	83608	C18orf21	19	6.79×10^{-09}
BIOGRID_INTERACTION	2805	GOT1	20	6.05×10^{-06}
BIOGRID_INTERACTION	53820	RIPPLY3	13	1.56×10^{-05}
BIOGRID_INTERACTION	30815	ST6GALNAC6	13	2.30×10^{-05}
Pathways				
REACTOME_PATHWAY	R-HSA-6809371	Formation of the cornified envelope	19	2.93×10^{-10}
REACTOME_PATHWAY	R-HSA-6805567	Keratinization	19	6.62×10^{-07}
REACTOME_PATHWAY	R-HSA-6798695	Neutrophil degranulation	23	4.20×10^{-04}
REACTOME_PATHWAY	R-HSA-6799990	Metal sequestration by antimicrobial proteins	4	9.04×10^{-03}
REACTOME_PATHWAY	R-HSA-168256	Immune System	50	1.62×10^{-02}
Protein Domains				
SMART	SM01394	SM01394	9	4.64×10^{-07}
PFAM	PF01023	S-100/ICaBP type calcium binding domain	9	6.42×10^{-07}
INTERPRO	IPR013787	S100/CaBP-9k-type, calcium binding, subdomain	9	1.73×10^{-06}
SMART	SM01391	SM01391	10	7.27×10^{-05}
UP_KW_DOMAIN	KW-0732	Signal	96	1.73×10^{-04}

components, the cornified envelope, and keratinization.

2.3. Gene-gene network analysis

To understand the potential interactions between the DEDM and related genes, we utilized the GeneMANIA web interface to construct gene-gene networks and identify the genes that are functionally associated with the DEDM genes. The constructed gene-gene networks are depicted in Figs. 3 and 4, with Fig. 3 focusing on the downregulated DEDM genes and Fig. 4 focusing on the upregulated DEDM genes.

Fig. 3A shows the gene-gene network of the downregulated and hypermethylated DEDM genes in warts, and its functions were predicted to involve the response to type I interferon (FDR = 1.20×10^{-09}), response to virus (FDR = 1.20×10^{-09}), cellular response to type 1 interferon (FDR = 1.20×10^{-09}), regulation of viral genome replication (FDR = 8.19×10^{-8}), and negative regulation of viral process (FDR = 1.28×10^{-7}). Likewise, Fig. 3B shows the gene-gene network of the downregulated and hypomethylated DEDM genes in warts, and its functions were predicted to involve keratinization (FDR = 1.65×10^{-35}), epidermal cell differentiation (FDR = 3.82×10^{-31}), keratinocyte differentiation (FDR = 1.84×10^{-29}), skin development (FDR = 1.84×10^{-29}), and intermediate filament cytoskeleton (FDR = 7.04×10^{-08}).

Fig. 4A shows the gene-gene network of the upregulated and hypermethylated DEDM genes in warts, and their functions were predicted to involve extracellular matrix structural constituent (FDR = 4.48×10^{-06}), growth factor binding (FDR = 4.48×10^{-06}), extracellular matrix organization (FDR = 8.55×10^{-04}), collagen-containing extracellular matrix (FDR = 2.29×10^{-02}), and cellular response to transforming growth factor beta stimulus (FDR = 4.86×10^{-02}). Similarly, Fig. 4B shows the gene-gene network of the upregulated and hypomethylated DEDM genes in warts. None of the predicted functions of these DEDM genes were statistically significant (FDR >0.05).

2.4. Protein-protein interaction (PPI) network analysis

To identify the predicted protein interactions of the DEDM genes, we used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database to construct a PPI network. Fig. 5 shows the PPI network of DEDM genes at a high confidence (0.7). Using

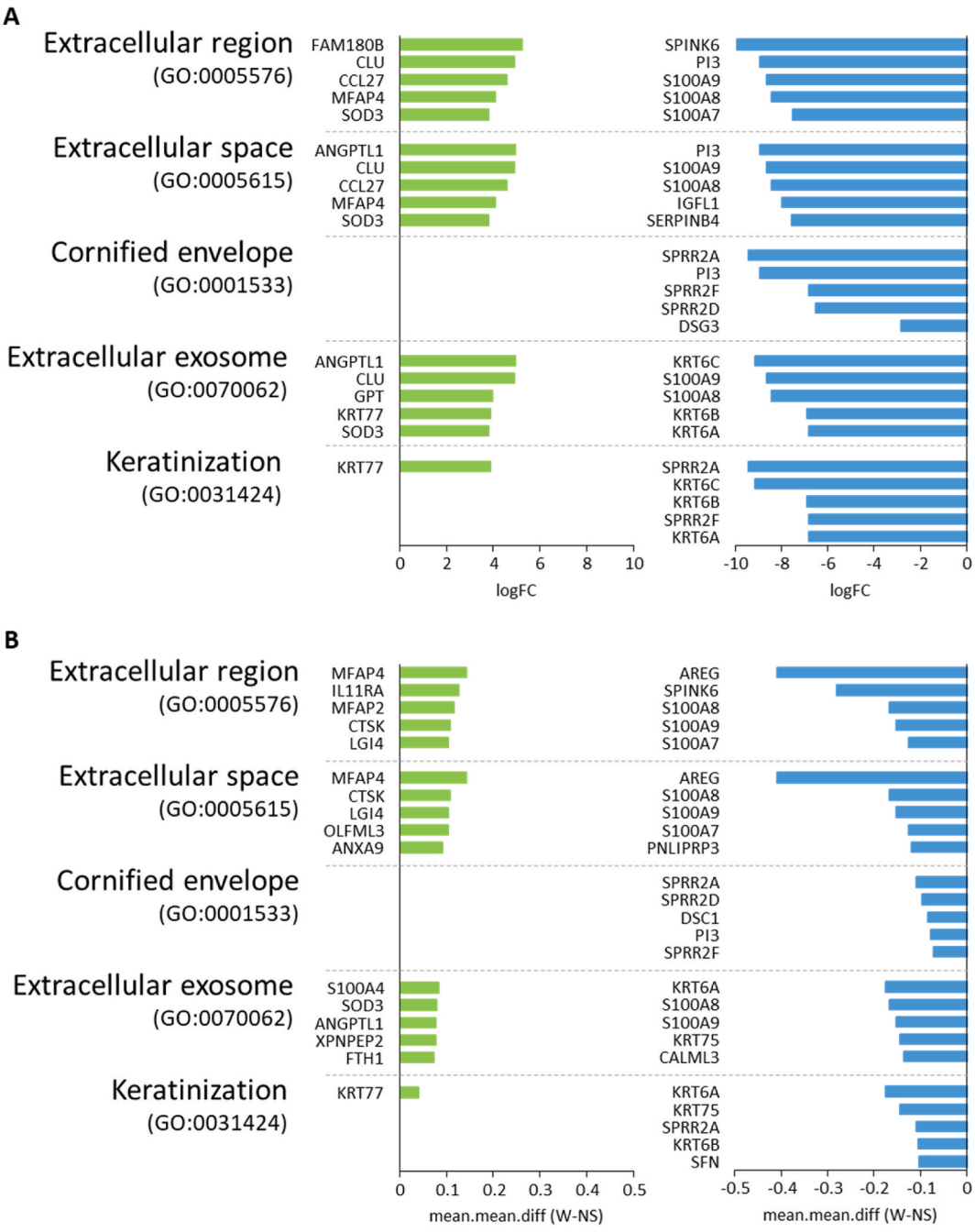

Fig. 2. Analysis of the top five most significant GO terms obtained for the DEDM genes in terms of expression (A) and methylation (B).

the Cytoscape application CytoHubba, the top hub genes in this PPI network were revealed to be elastin (*ELN*) (n = 10), integrin subunit beta 1 (*ITGB1*) (n = 9), TIMP metallopeptidase inhibitor 1 (*TIMP1*) (n = 6), matrix metallopeptidase 2 (*MMP2*) (n = 5), galectin 3 (*LGALS3*) (n = 5), collagen type I alpha 1 chain (*COL1A1*) (n = 5), and membrane alanyl aminopeptidase (*ANPEP*) (n = 5) genes.

2.5. Signaling network analysis

Lastly, we utilized the Signaling Network Open Resource 2.0 (SIGNOR 2.0) to carry out a signaling network analysis of the DEDM genes. Fig. 6 shows the signaling network generated from the DEDM genes with the highest confidence (0.9). The *ITGB1* gene had the highest number of interactions (n = 6).

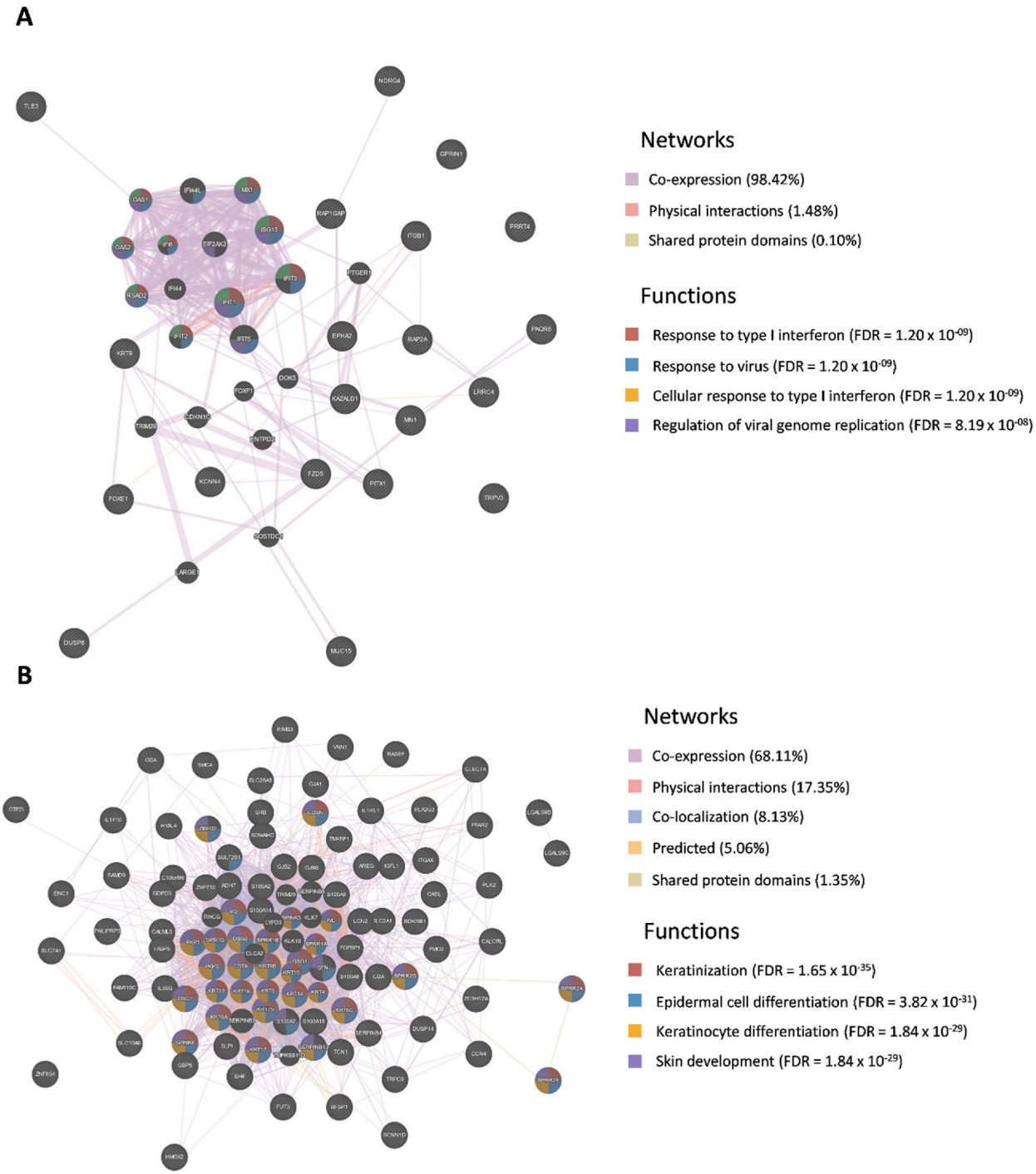

Fig. 3. Gene-gene networks of the downregulated and hypermethylated (A) as well as the downregulated and hypomethylated (B) DEDM genes in warts. *The size of a circle is proportional to the number of correlations with other genes in the gene-gene network. Striped circles represent the DEDM genes, while unstriped circles indicate associated genes.*

3. Discussion

Extracellular components, along with the cornified envelope and keratinization, were the most enriched GO terms among DEDM genes in common warts. Previous studies on diseases associated with high-risk HPV infection showed that these GO terms were similarly enriched (Table 3). This is unsurprising, as papillomaviruses are the only viruses known to begin their infection process at an

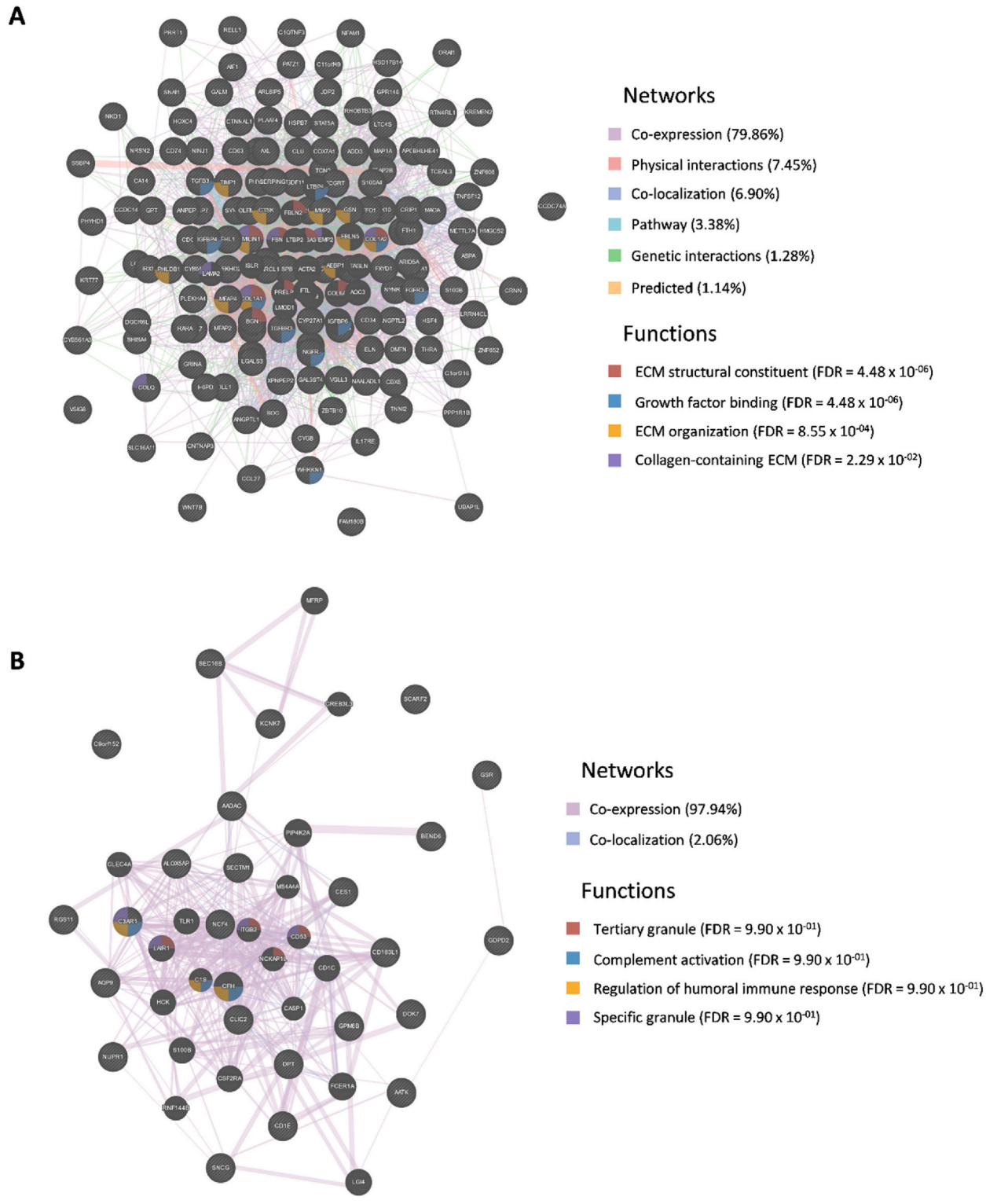

Fig. 4. Gene-gene network of the upregulated and hypermethylated (A) as well as the upregulated and hypomethylated (B) DEDM genes in warts. *The size of a circle is proportional to the number of correlations with other genes in the gene-gene network. Striped circles represent the DEDM genes, while unstriped circles indicate associated genes.*

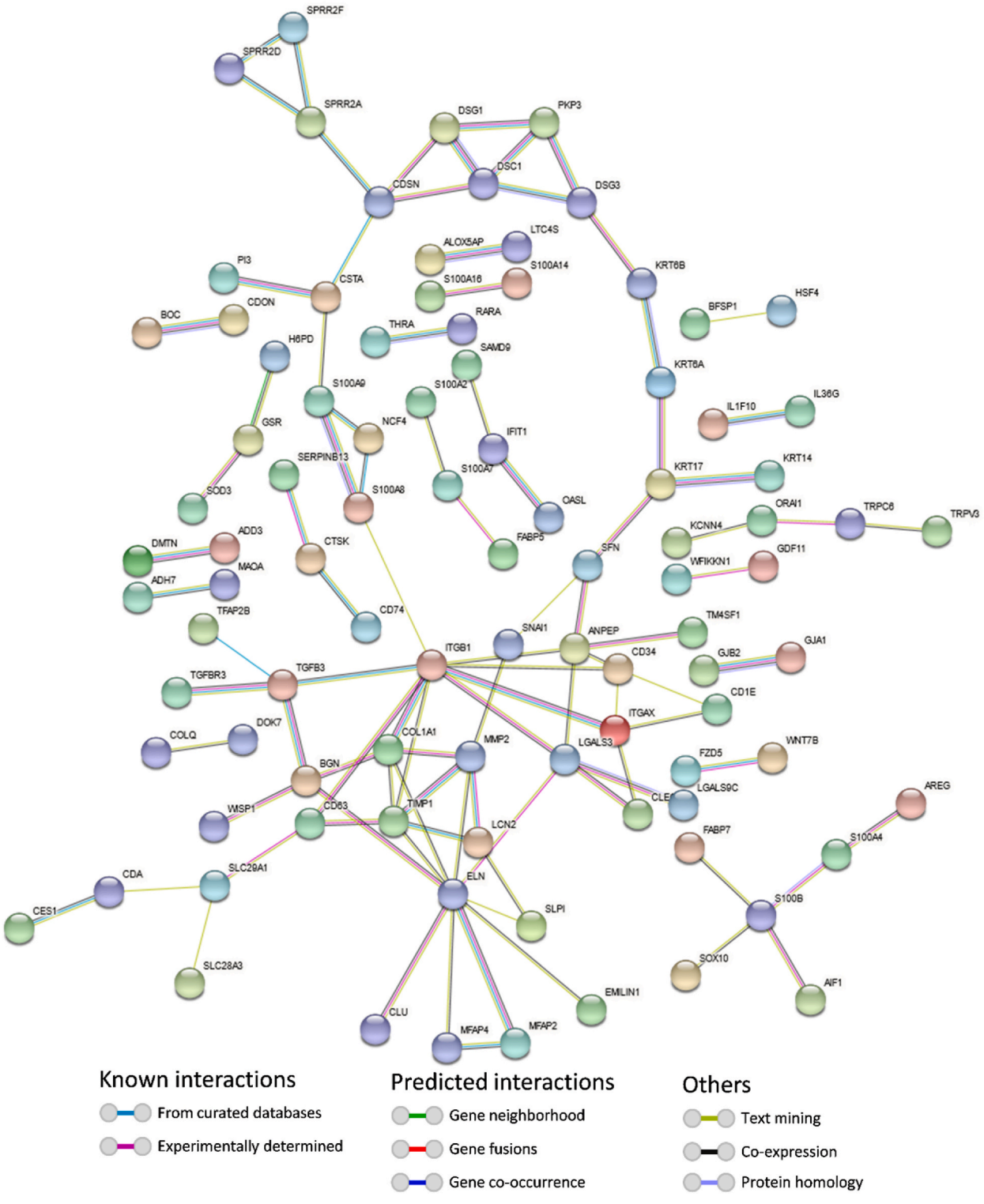

Fig. 5. Protein-protein network of DEDM genes in warts. *The interaction score was set at the highest confidence (0.900), and disconnected nodes are not shown.*

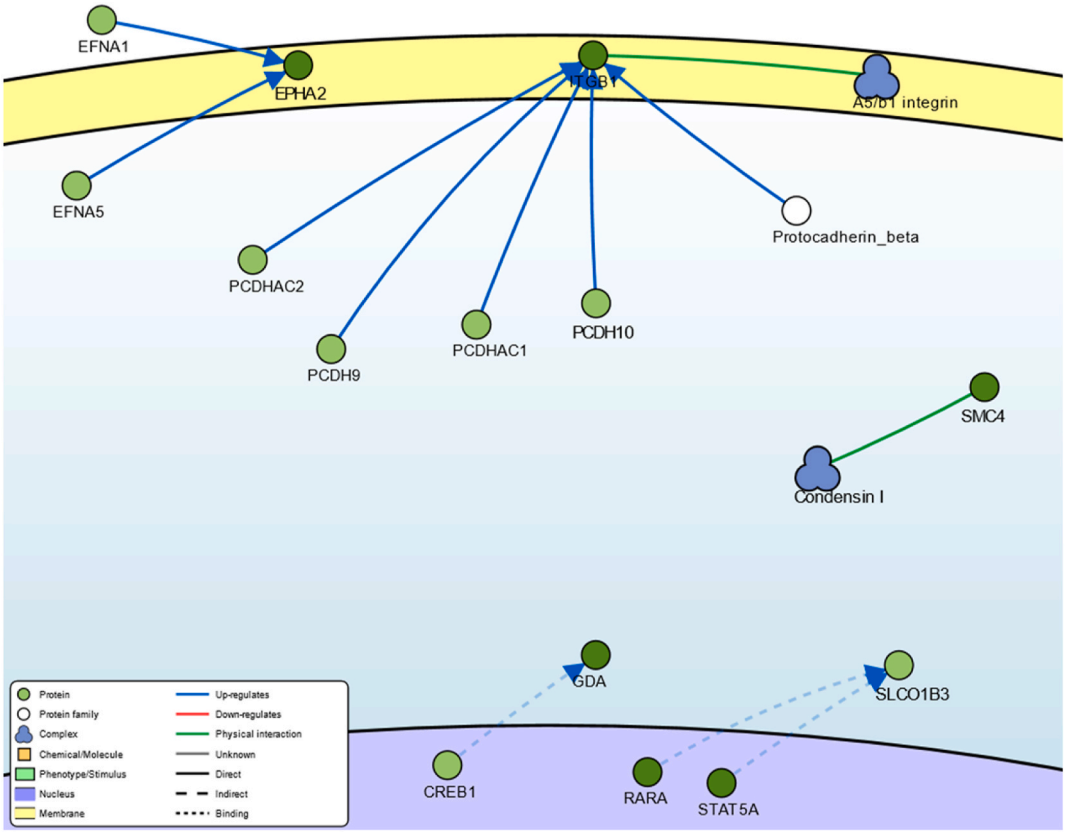

Fig. 6. Signaling network of DEDM genes in warts. *The interaction score was set at the highest confidence (0.9).*

extracellular location [12]. In fact, extracellular events play a key role in the entry of HPV virions into squamous epithelial cells via the basement membrane, which is composed of thin layers of ECM and is the primary site of HPV attachment [13–15]. However, it is important to note that the studies listed in Table 3 involved high-risk HPV infection, limiting the applicability of those findings to the present study, which focused on low-risk HPV infection.

Our findings revealed that the upregulated and hypermethylated DEDM genes in common warts were predicted to function as ECM structural and organizational components (Fig. 4A). In vitro, papillomaviruses have been shown to gain infectious entry into cells by binding to ECM components secreted by keratinocytes [26]. The ECM plays a critical role in tissue homeostasis and repair, and exploitation of the ECM allows HPV to enter basal keratinocytes [27]. Interestingly, genes encoding essential ECM components were among those identified as DEDM in common warts, including annexin a9 (*ANXA9*), collagen type I alpha 1 (*COL1A1*), elastin (*ELN*), integrin beta-1 (*ITGB1*), galectin-3 (*LGALS3*), and matrix metalloproteinase-2 (*MMP2*).

This study showed that the *ELN* gene was the top hub gene among the DEDM genes. *ELN* encodes the critical skin protein elastin, one of two components that make up the elastic fibers that confer elasticity and extensibility to the skin [28]. Elastic fibers are a major feature of the ECM and have been shown to be degraded by *MMP2* induction in ligamentum flavum hypertrophy [29]. On the contrary, elastin-derived peptides were reported to upregulate *MMP2* activation and subsequently enhance melanoma cell invasion [30].

In the present study, *MMP2* was identified as a DEDM gene in common warts that was also a hub gene. MMP family proteins are calcium-dependent zinc-containing endopeptidases which function in remodeling and breakdown of the ECM under normal conditions and pathological ones [31]. In the context of HPV infection, high-risk HPV types have been reported to possibly upregulate *MMP2* expression in lung adenocarcinomas and cervical cancer cells [32,33]. Likewise, certain S100 proteins such as S100A8, S100A9, and S100A14 - which are also DEDM in the present study, have been reported to promote cell motility and invasiveness by modulating *MMP2* expression [34–36].

Furthermore, the S100/ICaBP type calcium binding domain was shown to be the most enriched protein domain among the 276 DEDM genes in common warts. Although their functions are not well elucidated, the S100 proteins are known to have calcium and zinc-binding properties and tissue-specific expression patterns [37]. Various S100 proteins have been reported to be involved with diseases associated with high-risk HPV infection, as can be seen from Table 4.

Likewise, *LGALS3* had the second highest number of protein-protein interactions with other DEDM genes. The *LGALS3* gene encodes the β-galactoside-binding lectin galectin-3, which is abundantly expressed in the cytoplasm of normal keratinocytes and is involved in inflammatory skin diseases, HPV-positive head and neck squamous cell carcinoma, and HPV-induced warts [54–56]. A previous study focusing on retinal pigment epithelial cells showed that extracellular galectin-3 is a positive regulator for the clustering

Table 3
Previously reported associations of the top five most significant GO terms obtained for DEDM genes in common warts with other HPV-related diseases.

Study samples	Ref.	HPV type	GO:0001533	GO:0005576	GO:0005615	GO:0031424	GO:0070062
Cervix							
Cervical cancer	[16]	n/a	✓				✓
Cervical pre-cancer lesions	[17]	n/a	✓	✓	✓	✓	
Cervical squamous cancer	[18]		✓	✓	✓		✓
Cervical squamous cell carcinoma	[19]	n/a		✓	✓		✓
Cervical squamous cell carcinoma	[18]	n/a	✓	✓	✓		✓
Head and neck							
Head and neck squamous cell carcinoma	[20]	n/a		✓	✓		✓
Head and neck squamous cell carcinoma	[21]	n/a		✓	✓		✓
Head and neck squamous cell carcinoma	[22]	High risk			✓	✓	✓
Murine model							
K14E6/K14E7 transgenic mice	[23]	High risk		✓	✓	✓	
Vulva							
HPV-transformed primary cell lines from neoplastic vulval lesions	[24]	High risk	✓	✓	✓	✓	
Vulvar squamous cell carcinoma	[25]	High risk		✓			✓

GO:0001533 – *cornified envelope* - A type of plasma membrane that has been modified through addition of distinct intracellular and extracellular components, including ceramide, found in cornifying epithelial cells (corneocytes).
GO:0005576 – *extracellular region* – The space external to the outermost structure of a cell.
GO:0005615 – *extracellular space* – That part of a multicellular organism outside the cells proper, usually taken to be outside the plasma membranes, and occupied by fluid.
GO:0031424 – *keratinization* – The process in which the cytoplasm of the outermost cells of the vertebrate epidermis is replaced by keratin.
GO:0070062 – *extracellular exosome* – A vesicle that is released into the extracellular region by fusion of the limiting endosomal membrane of a multivesicular body with the plasma membrane.

of CD147 and ITGB1 [57].

ITGB1, a broadly expressed cell surface receptor, was identified by signaling network analysis as a hub gene and forms a part of the fibronectin receptor integrin α5β1, which is used by different viruses to attach and gain entry into host cells [58,59]. *ITGB1* also acts as an upstream regulator of caveolin-1, a scaffolding protein that facilitates viral entry into host cells [60]. In fact, caveolin-1 facilitates HPV31 entry into human keratinocytes and is upregulated by HPV16 [61,62]. In a murine model, blocking ITGB1 function decreased epidermal hyperproliferation and thickness, but another study showed that ITGB1 deletion converts the airway epithelium from a monolayer to a multilayer [63,64].

Our findings show that palmoplantar keratoderma (PK) is the most enriched disease among DEDM genes, largely due to the overlap of the gap junction and keratin genes. PK refers to a heterogeneous group of skin conditions that cause excessive thickening of the epidermis of the palms and soles. PK can be acquired through environmental factors - including HPV infection - or it can be hereditary [65]. Epidermodysplasia verruciformis, an inherited disorder with PK as a feature, is characterized by high susceptibility to infection by certain HPV types [2]. Interestingly, 19 of the DEDM genes in the current study overlapped with those significantly expressed in pachyonychia congenita, a hereditary skin disorder characterized by painful plantar keratoderma and thickened toenails [66].

There are several limitations to our findings that merit exploration in future studies. The sample size used in the microarray datasets is relatively small (n = 12), which might prevent the extrapolation of our findings to populations outside of the datasets. However, it is worthwhile to note that some strengths of the utilized datasets are that the cohort was all male (which reduces gender-specific variation) and that the normal skin biopsies were taken from sites adjacent to the wart biopsies for most samples (which reduces inter-organ variation). Moreover, as our study focused on an all-male cohort, it would be interesting to explore the impact of HPV infection on host gene expression and DNA methylation in the prostate of males and breast tissue of females, as previous studies have revealed possible links between HPV infection and certain cancers of the prostate and breast [67–69]. Furthermore, the utilized datasets did not include histological analysis of the wart samples, meaning that their gene expression and DNA methylation profiles contain a combination of expression patterns from the basal and suprabasal layers of the epidermis. Lastly, future studies would be useful to re-validate our findings by qPCR analysis in a separate cohort of samples.

4. Conclusions

In the present study, an integrative analysis of the gene expression and DNA methylation profiles of common warts was carried out. Our findings showed that 52% of DEDM genes were both upregulated and hypermethylated, with extracellular components being the most enriched GO terms among DEDM genes. Unsurprisingly, genes encoding essential extracellular components were among those that were DEDM, including *ELN* and *MMP2*, which were also found to be significant hub genes. However, there were also several DEDM genes with functions that have not been well-elucidated in previous studies. It is important to note that the extrapolation of our

Table 4
Role of S100 proteins in HPV-associated and other diseases.

Type	DEDM in the current study?	May be associated with	Ref.
S100 proteins	n/a	Replication stage of HPV infection in HPV-positive cervical stroma	[38]
S100A2	**Yes**	Progression of human prostate adenocarcinoma	[39]
S100A4	**Yes**	Progression of human prostate adenocarcinoma	[39]
S100A7	**Yes**	Prognosis of HPV-positive base of tongue squamous cell carcinoma	[40]
S100A8	**Yes**	Onset and progression of HPV-positive oral squamous cell carcinoma	[41]
S100A8/A9	**Yes**	Induction of granulocyte chemotaxis into HPV8-infected epidermodysplasia verruciformis lesions	[42]
		Regulation of HPV16 E7 phosphorylation mediated by casein kinase II	[43]
S100A9	**Yes**	Maintenance of the differentiated state of the epithelium, with S100A9 downregulation associated with increased susceptibility to esophageal squamous cell carcinoma tumors	[44]
S100A10	No	Trafficking of HPV16 particles into host cells via an interaction with HPV16 and annexin	[45]
S100A11	No	Proliferation, migration, invasion, and epithelial-mesenchymal transition of cervical cancer cells	[46]
S100A12	No	Prognosis of HPV-negative hypopharyngeal squamous cell carcinoma	[47]
S100A14	**Yes**	Prognosis of oral squamous cell carcinoma	[48]
		Progression of epithelial ovarian tumors	[49]
		Regulation of proliferation, migration, and invasion in cervical cancer cells	[49]
S100A16	**Yes**	Involvement in cancer stem-like cells	[50]
		Proliferation, migration, and tumor angiogenesis of HeLa cells via the regulation of the PI3K/AKT signaling pathway	[51]
S100B	**Yes**	Putative therapeutic target of neurological disorders, inflammatory bowel disease, obesity, diabetes, and cancer	[52]
S100P	No	Contribution to the outgrowth of aggressive tumor cells that are resistant to cytotoxic therapy	[53]

findings is limited by the microarray datasets' relatively small and all-male cohort. Future studies on common warts are needed to corroborate our findings in a larger population.

5. Materials and methods

5.1. Microarray datasets

The DNA methylation and gene expression datasets of HPV-induced common warts were retrieved from the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO). GSE213888 included the methylation data from 12 common warts and 12 matched controls from Jordanian males, while GSE136347 contained the gene expression data from the same samples and cohort. The inclusion criteria for the datasets were being male and clinical diagnosis of common warts, while the exclusion criteria were the presence of any comorbid autoimmune or dermatological conditions [70,71].

5.2. Identification of DEDM genes

Differentially methylated (DM) genes between warts and normal skin were calculated using the R package RnBeads. Basically, the methylation level of genes was calculated and given a ranking score using 3 criteria: mean β difference between warts and normal skin, relative effect sizes (log2 of the quotient in methylation), and AP. The worst scores of these 3 measurements were combined into one single score called "combined ranking score" as described previously [72]. The top 3000 genes with the lowest "combined ranking score" were selected. The selected 3000 differentially methylated (DM) genes had an AP=<0.04, mean β difference >0.03 and < −0.03 and $\log_2$ of the quotient with a maximum value of 1.686 and a minimum value of −1.751.

On the other hand, differentially expressed (DE) genes were calculated using edgeR version 3.22.3 package [73]. Adjusted p-value (AP) < 0.05 and absolute fold change (|FC|) >2 were set as the threshold for DE genes. Using these cut-off measurements, a total of 3140 genes were identified as DE genes in warts compared to normal skin samples.

To explore the relationship between DNA methylation and gene expression, the DM genes were matched to the DE genes, and only the overlapped genes, i.e., both differentially expressed and differentially methylated (DEDM) genes, could be selected for further analysis.

5.3. Enrichment analysis of DEDM genes

The functional annotation of DEDM genes was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). DAVID is a web server that provides functional annotation and enrichment tools to facilitate the biological interpretation of large lists of genes. It collects information on the annotations of species-specific gene/protein identifiers from public genomic resources such as Uniprot, DisGeNET, Gene Ontology, BioGRID, Reactome, and KEGG [74,75].

5.4. Network construction and analysis

A gene-gene network of DEDM genes was constructed using the GeneMANIA web interface. GeneMANIA identifies the genes that are related to the query genes through its very large functional association dataset, allowing users to identify new members of pathways or complexes [76].

A protein-protein interaction (PPI) network was created using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 11.5. The aim of the STRING database is to gather, score, and integrate all publicly accessible data on known and predicted protein-protein interactions [77].

After inputting the genes into STRING, CytoHubba was utilized to choose the hub genes in the PPI network based on the connection degree. CytoHubba is a Cytoscape application that predicts significant hub genes in a biological network through topographical analysis [78].

Lastly, a signaling network was constructed using the Signaling Network Open Resource 2.0 (SIGNOR 2.0). SIGNOR 2.0 collects signaling information from the scientific literature and stores the data as binary causative relationships between biological entities that can be represented in a graphical format [79].

Author contribution statement

Mansour A Alghamdi, Laith N. AL-Eitan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Amneh H. Tarkhan: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at "the NCBI Gene Expression Omnibus database" under the accession number GSE136347 and GSE213888, respectively.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board (IRB) at Jordan University of Science and Technology (JUST) (Ref. 19/105/2017). All procedures performed in this study were in compliance with the IRB's human research ethics protocol and were performed at King Abdullah University Hospital in Irbid, Jordan. All recruited participants were provided written informed consent.

Consent for publication

All authors give their consent for the publication of the manuscript.

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.

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