Clinics of Oncology

Research Article ISSN: 2640-1037 | Volume 7

IGF2BP3 Is Associated with HPV Status and Tight Junction in HPV-Related Cervical Cancer

Han X[1], Wu X[1], Gong A[2*] and Wang L[1*]

[1]Center of Clinical Laboratory, Dushu Lake Hospital Affiliated to Soochow University, Medical Center of Soochow University, Suzhou Dushu Lake Hospital, Soochow University, Suzhou, 215123, China.

[2]Department of Cell Biology, School of Medicine, Jiangsu University, Zhenjiang 212013 Jiangsu, China

[*]Corresponding author:

Lin Wang,
Center of Clinical Laboratory, Dushu Lake Hospital Affiliated to Soochow University, Medical Center of Soochow University, Suzhou Dushu Lake Hospital, Soochow University, Suzhou, 215000, China,
Aihua Gong, Department of Cell Biology, School of Medicine, Jiangsu University, Zhenjiang 212013 Jiangsu, China

Received: 01 Aug 2023
Accepted: 11 Sep 2023
Published: 19 Sep 2023
J Short Name: COO

Keywords:

N6-methyladenosine; IGF2BP3; Human papillomavirus; Cervical squamous cell carcinoma; Tight junction

Citation:

Wang L, IGF2BP3 Is Associated with HPV Status and Tight Junction in HPV-Related Cervical Cancer. Clin Onco. 2023; 7(1): 1-10

Abbreviations:

IGF2BP3: insulin like growth factor 2 mRNA binding protein 3; HPV: human papillomavirus; CESC: cervical squamous cell carcinoma; m6A: N6-methyladenosine; IGF2BP2: insulin like growth factor 2 mRNA binding protein 2; TCGA: The Cancer; Genome Atlas; CETx: Genotype-Tissue Expression; TJ: tight junction; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; ACC: adenoid cystic carcinoma; BLCA: bladder cancer; TIMER: Tumor IMmune Estimation Resource; CLIN: cervical intraepithelial neoplasia; DEGs: differentially expressed genes; ECM: extracellular matrix; GSEA: Gene Set Enrichment Analysis; TJP1: tight Junction Protein 1; TJP2: tight Junction Protein 2; CLDN11: Claudin 11; CLDN12: Claudin 12; MARVELD2: MARVEL [Membrane-Associating] Domain Containing 2; MARVELD3: MARVEL [Membrane-Associating] Domain Containing 3, OCLN: Occludin; MAGI-1: Membrane-Associated Guanylate Kinase, WW And PDZ Domain-Containing Protein 1; MAGI2: Membrane-Associated Guanylate Kinase, WW And PDZ Domain-Containing Protein 2; LAMA3: Laminin Subunit Alpha 3; LAMC2: Laminin Subunit Gamma 2; ITGB4: Integrin Subunit Beta 4; TJAP1: Tight Junction Associated Protein 1; ZO-2: Zona Occludens 2 also known as TJP2; MDCK: Madin-Darby canine kidney

1. Abstract

1.1. Background: N6-methyladenosine [m6A] is the most prevalent type of RNA modification of eukaryotic mRNAs. It is known to have broad effects on malignant tumors by acting as oncogenes or anti-oncogenes, and participating in tumor proliferation, tumorigenesis, differentiation, metastasis and invasion. However, the effects of m6A modification on in cancers have been rarely investigated. In this study, we performed integrative analysis to investigate the function of IGF2BP3, an essential regulator of m6A in the invasion and transmission of HPV in cervical squamous cell carcinoma [CESC]. We hypothesized that the tumors will express high level of IGF2BP3 and that this is probably associated with a poor prognosis in CESC.

1.2. Methods: We investigated the expression levels of IGF2BP3 and its prognostic value in CESC in multiple datasets including the TCGA, GTEx and TIMER. GO and KEGG analyses of differentially expressed genes were performed using GOplot, org.Hs.eg.db and clusterProfiler packages and visualized using the ggplot2 package in R language.

1.3. Results: In HPV-positive tumors, IGF2BP3 was positively associated with tumor HPV status. Further analysis demonstrated that the high levels of IGF2BP3 were correlated with signaling pathways related to cell-cell and cell-extracellular matrix [ECM] interactions. These interactions participated in receptor ligand ac-

tivity, neuroactive ligand receptor interaction and chemical carcinogenesis receptor activation. Moreover, correlation analysis showed that IGF2BP3 would impair the formation of cell polarity and cell-cell contacts by dysregulating the expression of tight junction molecules.

1.4. Conclusions: Analysis demonstrated that high expression levels of IGF2BP3 were associated with HPV status and were predictive of a poor prognosis. In addition, we demonstrated that IGF2BP3 serves as a mediator of tight junction formation in HPV-associated CESC by regulating the function of cell-cell and cell-ECM interactions. Collectively, our analysis provided a promising target for the development of noval anti-cancer therapeutics.

2. Introduction

Cervical cancer is the fourth most common cancer among women globally; global analysis estimated that there would be 604 000 new cases and 342 000 deaths in 2020 [1]. Approximately 90% of the new global cases and deaths in 2020 occured in low- and middle-income nations [2]. The development of cervical cancer is closely associated with strains of the human papillomavirus [HPV] infection [3]. In general, most HPV infections are transient; and are probably cleared by the immune system within 6 - 18 months [4]. However, some patients with continuous infection may develop premalignant lesions and cancer as a consequence of serval risk factors, including age, immunodeficiency, smoking, oral contraceptives and chlamydia trachomatis [5, 6]. Several factors have been showed to be involved in the development and progression of cervical squamous cell carcinoma [CESC], including genetic and epigenetic alterations, abnormalities in key cellular signaling pathways, the cervicovaginal microbiome, and immune escape [7, 8]. Although countless efforts have attempted to identify new options for CESC, the prognosis and postoperative survival of patients with CESC are not yet accepteable. Furthermore, the soecific pathogenic mechanism underlying the developments of CESC have yet to be fully elucidated; consequently, there is a significant need for the identification and development of therapeutic targets for CESC.

Over recent years, there has been a significant increase in number of studies investigating the link between epigenetics and carcinogenic mechanisms [9]. N6-methyladenosine [m6A] is the most prevalent type of RNA modification of eukaryotic mRNAs [10]. It is well accepted that m6A plays an important role in many biological processes, including tissue development, the differentiation of naive pluripotency stem cells, heat shock response and DNA damage [11]. m6A is also known to be involved in many cellular RNA processes, such as transcription, splicing, translation, nuclear transport and degradation [12, 13]. There are several known regulators of m6A, including ‘writers’, ‘erasers’ and ‘readers’; these execute the installation, demethylation and recognition of m6A, respectively [13]. Previous research showed that the writer methyltransferase installs m6A methylation on target RNAs [14] and that erasers catalyze the removal of m6A on RNAs [15]. Readers with recognization functionality have also been shown to bind to m6A-RNAs to control their fate [16]. Given the growing body of evidence indicating that m6A modification is involved in the homeostasis of hosts and microbes [mostly viruses and bacteria] [17-21], we hypothesized that m6A or m6A regulators would participate in HPV-related CESC. Previous studies have demonstrated that m6A regulators are associated with HPV status and the immunosuppressive microenvironment of HPV-related cancers [22, 23]. A previous study determined that m6A modifications of circE7 led to preferential localization to the cytoplasm, associated with polysomes, and then translation to produce E7 oncoprotein. This finding provided novel insights into how HPV regulates infection and tumorigenesis [24]. In addition, E6/E7 have been linked to the promotion of aerobic glycolysis and the progression of CESC by regulating MYC methylation sites by activating IGF2BP2 [25]. However, the specific relationship between HPV and m6A methylation has yet to be eluciated. Furthemore, it is important to gain enhanced knowledge of the mechanism underlying how m6A plays a role in the progression of HPV-induced CESC.

Here, we investigated the expression patterns, prognostic relevance, and correlations of IGF2BP3, an established regulator of m6A, with HPV status. We found that IGF2BP3 expression levels were associated with HPV status and a poor prognosis by investigating paired specimens from the The Cancer Genome Atlas Program [TCGA], Genotype Tissue Expression [GTEx] datasets. Previous studies have showed that some viruses are able to hijack the different components of tight junctions [TJs] to initiate their infections by impairing the formation of cell polarity and cell-cell contacts [26-28]. Hence, we used correlation analysis to investigate the association between IGF2BP3 and TJ proteins. We hope to identify promising targets for the development of noval anti-cancer therapeutics by targeting IGF2BP3 and tight junction barriers in CESC.

3. Materials and Methods

3.1. Sample Data Acquisition

Integrated expression profile and clinical features were obtained from the TCGA and GTEx datasets. Raw data were downloaded from UCSC Xena [https://xena.ucsc.edu/]; data relating to 305 and 10 samples were downloaded from TCGA and GTEx data, respectively. The CESC data, companied with detailed HPV infection status, were used to investigate the correlation between IGF2BP3 expression and HPV infection [29]

3.2. Enrichment Analysis

The samples were ranked according to IGF2BP3 expression and divided into IGF2BP3-high and IGF2BP3-low expression group. The top 50% of patients were in the IGF2BP3-high group; the remaining patients were classified into the IGF2BP3-low group.

The DESeq2 [v1.26.0] package in R was used to identify differentially expressed genes [DEGs] in the two groups [30]. The significance thresholds for DEGs were adjusted [Adj] P value <0.05 and |logFC| >2. Gene ontology [GO] and Kyoto Encyclopedia of Genes and Genomes [KEGG] analyses of DEGs were performed using GOplot, org.Hs.eg.db and clusterProfiler packages and then visualized using the ggplot2 package [31]. Gene Set Enrichment Analysis [GSEA] was also performed to investigate the potential mechanisms related to the signature genes.

3.3. Statistical Analysis

Data are presented as mean ± standard deviations [SD]. All statistical analyses were performed in GraphPad Prism version 8.0.2. Survival analysis was performed by Kaplan-Meier curve and tested by the log-rank test. Spearman's correlation analysis was used to evaluated correlations between two key parameters. The two experimental groups were compared by the Student's t-test for unpaired data. $P < 0.05$ was considered significant. P values are indicated as *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

4. Results

4.1. Expression Profiles of IGF2BP3 in CESC and Clinical Relevance

We investigated the expression profiles of IGF2BP3 in pan-cancer, including adenoid cystic carcinoma, bladder cancer, CESC and so on, across all TCGA tumors by Tumor IMmune Estimation Resource [TIMER] (Figure S1). As shown in (Figure S1), IGF2BP3 was over-expressed in multiple tumor tissues, for example, bladder urothelial carcinoma, esophageal cancer, cholangiocarcinoma and CESC. To further verify the expression profile of IGF2BP3 in CESC, we compared the expression levels between tumors and adjacent normal tissues in the TCGA and GTEx datasets. The expression of IGF2BP3 was higher in tumors [n=305] than in normal tissues [n=10] in CESC (Figure 1A) [P <0.0001]. The contribution of IGF2BP3 to the survival of patients with CESC is given in a survival map (Figure 1B). Then, we plotted survival probability against time [days] and generated two curves: blue for low expression levels of IGF2BP3 and red for high levels of IGF2BP3. The overall survival [OS], disease-specific survival [DSS] and progression free interval survival [PFS] curves of the two cohorts overlapped to some extent [P > 0.05], indicating the expression levels of IGF2BP3 was not associated with the survival of patients. ROC curve analysis showed that IGF2BP3 levels could representent a significant predictor of CESC [AUC > 0.8] (Figure 1C), thus indicating that IGF2BP3 was associated with a poor prognosis in CESC patients.

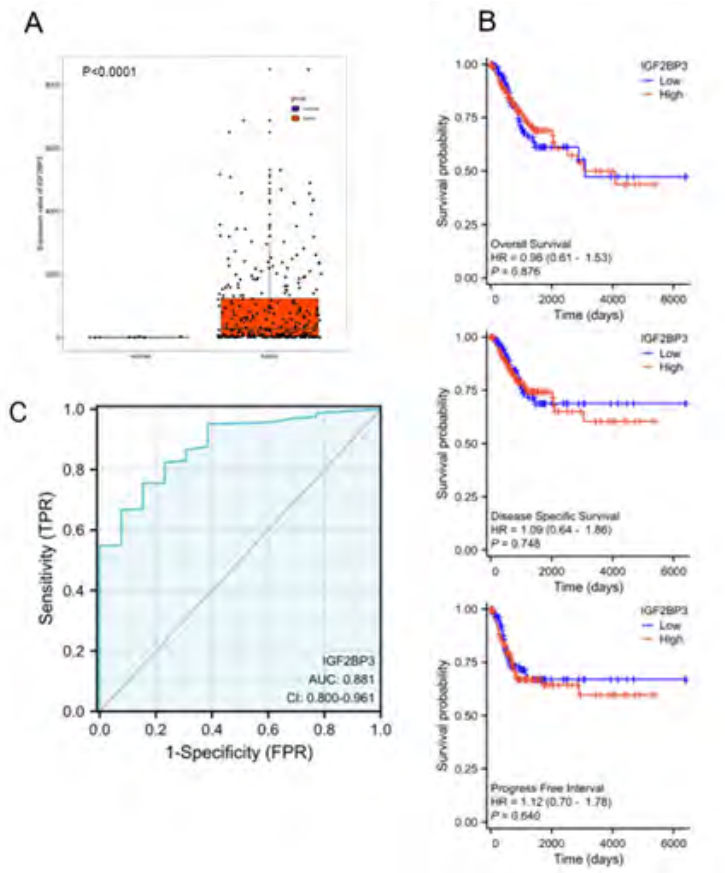

Figure 1: Expression pattern of IGF2BP3 in CESC and clinical relevance.
A: The expression of IGF2BP3 in CESC and normal tissue;
B: Kaplan-Meier curve of IGF2BP3 in CESC;
C: The ROC curve of IGF2BP3 in CESC patients.

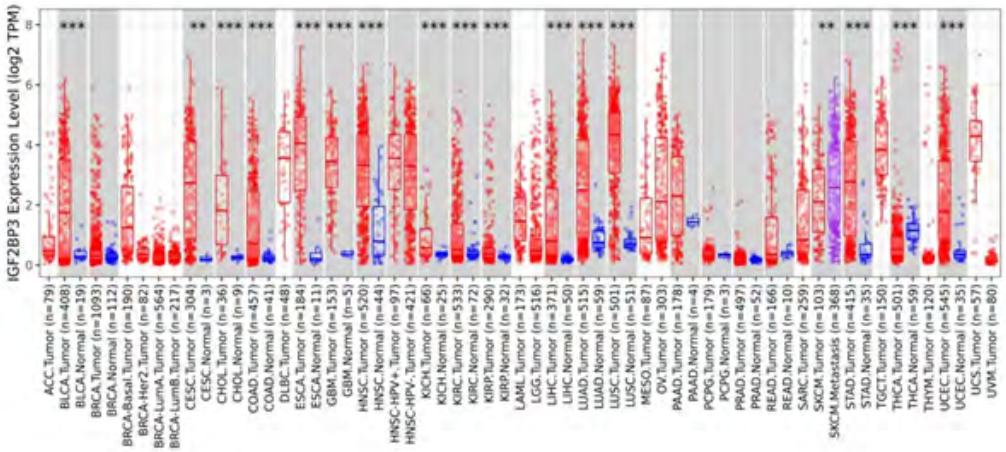

Figure S1: The expression profile of IGF2BP3 in pan-cancer, the expression profile of IGF2BP3 in pan-cancer across all TCGA tumors using TIMER 2.0. IGF2BP3 was over-expressed in multiple tumor tissues including CESC.

4.2. IGF2BP3 was Associated with HPV Infection in HPV Related CESC

HPV infection is considered to be a causative factor for malignant cervical lesions [32]. Next, we aimed to identify the specific relationship between IGF2BP3 expression and HPV infection; for thus, we used CESC data and HPV infection status [29]. Analysis demonstrated that the expression levels of IGF2BP3 were significantly higher in patients with HPV infection when compared to those without HPV infection (Figure 2A) [$P < 0.05$]. Moreover, the levels of IGF2BP3 variy according to CLIN: e6_cat_k4 and CLIN: e6ratio_cat_k4. (Figure 2B). IGF2BP3 expression was higher in CLIN: e6_cat_k4_C2 and CLIN: e6ratio_cat_k4_C2. E6 category [32=C1, 33=C2, 31=C3, 32=C4], and the E6 ratio category [33=C1, 34=C2, 30=C3, 31=C4] [$P < 0.05$]

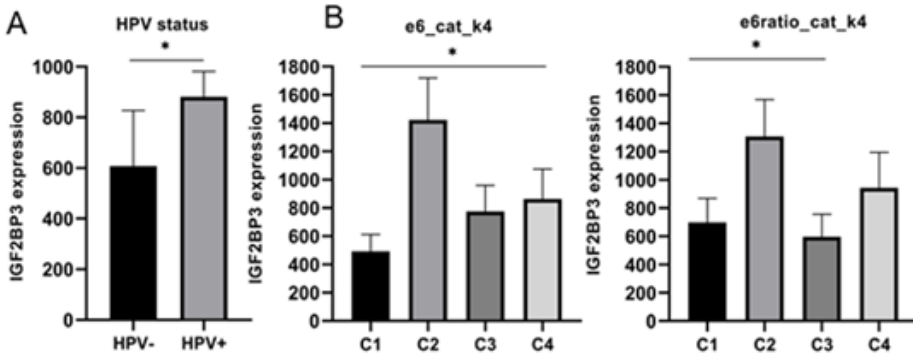

Figure 2: IGF2BP3 was associated with HPV infection in HPV related cancer

A: IGF2BP3 expression in the HPV -/+ group of CESC;

B-C: IGF2BP3 expression in different categories of CLIN: e6_cat_k4 (E6 category) and CLIN: e6ratio_cat_k4 (E6 ratio category). E6 category (32=C1, 33=C2, 31=C3, 32=C4), E6 ratio category (33=C1, 34=C2, 30=C3, 31=C4). C1-4 represent different E6 categories.

4.3. IGF2BP3 was Involved in Cell-Cell and Cell-ECM Interactions in HPV-Related CESC

In order to investigate the specific role of IGF2BP3 expression levels on HPV-associated CESC, we first ranked patients according to IGF2BP3 expression.The top 50% of patients were classfied into the IGF2BP3-high group, while the remaining patients were classified into the IGF2BP3-low group. Next, we performed GO and KEGG enrichment analysis using DEGs between the IGF-2BP3-high group and IGF2BP3-low group in CESC. GO analysis showed that the enrichment of DEGs was detected in several pathways related to intrinsic cell-cell contacts and cell-extracellular matrix [ECM] interactions. These pathways involve receptor ligand activity, signaling receptor activator activity, carboxylic acid binding and peptidase inhibitor activity (Figure 3A). In addition, KEGG analysis identified several differential enriched pathways, including neuroactive ligand receptor interaction, the calcium signaling pathway, chemical carcinogenesis receptor activation and retinol metabolism (Figure 3B). All of these pathways identified by GO/KEGG focused on receptor-ligand activity or molecular binding; these processes are known to play important roles in cell-cell or cell-ECM interactions [33]. Next, we performed Gene Set Enrichment Analysis [GSEA] in CESC to further investigate the

enrichment of DEGs in cell-cell or cell-ECM interactions. The DEGs were significantly associated with ECM organization, genes encoding structural ECM glycoproteins, transmission across chemical synapses, the suit of genes encoding the core extracellular matrix, the neuronal system, nuclear receptors meta-pathwaysm and neuroactive ligand-receptor interaction (Figure 3C). Thus, DEGs were highly associated with ECM formation and ligand-receptor interaction. Therefore, our data indicated that IGF2BP3 plays a key role in cell-cell contacts and cell-ECM interactions in HPV-related cancer.

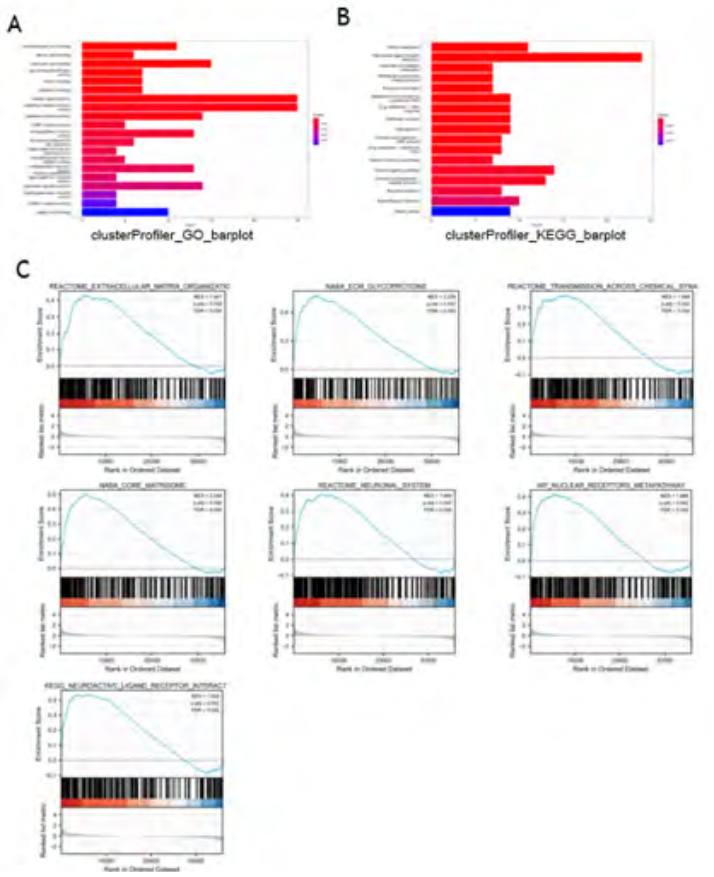

Figure 3: IGF2BP3 was involved in cell-cell and cell-ECM interactions in HPV-related CESC
A-B: GO and KEGG enrichment analysis of differential genes in CESC;
C: GSEA analysis of differential genes in CESC.

4.4. IGF2BP3 was Related to Tight Junction Formations in HPV-Related CESC

Our earlier enrichment analysis suggested that IGF2BP3 was associated with receptor ligand activity, extracellular matrix organization and transmission across chemical synapses, thus playing a key role in cell-cell contacts and cell-ECM interactions [34, 35]. Moreover, it has been confirmed that tight junctions are continusly associated with cell-cell contacts and cell-extracellular matrix interactions throughout plasticity in the endometrium [36, 37]. Accordingly, we analyzed the relationship between IGF2BP3 expression and TJs to investigate how IGF2BP3 might influence these interactions. We investigate the expression profile of 27 TJ molecules in CESC using TCGA data (Figure 4A) and identified 17 DEGs in CESC (Figure 4A). The expression levels of these 17 DEGs between normal and tumor tissues are shown in (Figure S2). The expression profiles of these TJs molecules were similar to those of IGF2BP3 in CESC, as determined by the co-expression heatmaps (Figure 4B). In addition, we used TCGA data to perform correlation analyses of the expression of the TJ molecules and IGF-2BP3 in CESC (Figure 4C). Then, patients were ranked according to IGF2BP3 expression levels: the top 50% of patients expressed high levels of IGF2BP3, while remaining patients expressed low levels of IGF2BP3. In CESC, we found that samples with high expression levels of IGF2BP3 also expressed higher levels of TJP1, TJP2, CLDN11, CLDN12, MARVELD2, MARVELD3, OCLN, MAGI2, LAMA3, LAMC2, ITGB4 and TJAP1.

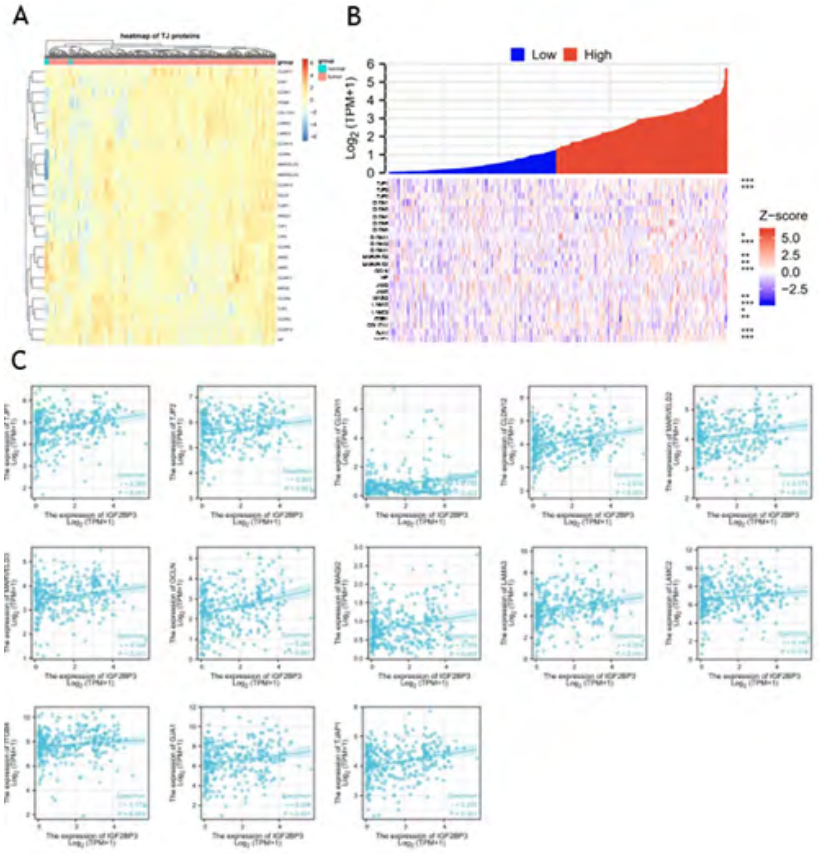

Figure 4: IGF2BP3 was related to the tight junctions in HPV-related CESC

A: The expression spectrum of IGF2BP3 in CESC and normal tissue;

B: The co-expression heatmap of IGF2BP3 with DEGs in CESC;

C: Correlation analysis of TJ molecules and METTL3 expression in CESC.

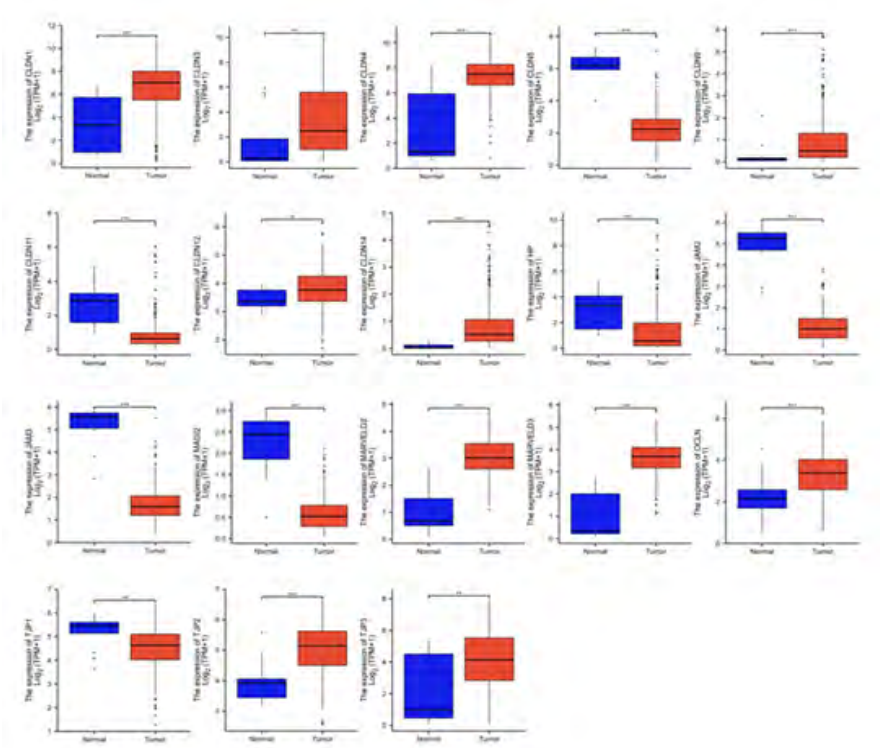

Figure S2: The expression of TJ molecules in CESC

A: Expression levels of the 17 differentially expressed TJ molecules between normal and rumor in CESC.

5. Discussion

m6A RNA modification is widely known to exert broad effects on malignant tumors by participating in tumor proliferation [38, 39], differentiation [40, 41], tumorigenesis [42], invasion and metastasis [43, 44], as either oncogenes [45, 46] or anti-oncogenes [47]. However, the specific effects of microbed on cancer formation, prognosis, and treatment have been rarely investigated. Due to the increasing evidence for the involvement of HPV in cancer, the diagnosis and treatment of microbe-related cancer has improved notably over recent years [48, 49]. There is an urgent need to develop new therapeutic approaches for HPV-related cancers; this will require the discovery of precise and effective targets related to HPV invasion [50] and DNA viruses' transmission [51]. In the present study, we revealed that IGF2BP3, one of the main m6A regulators, was closely associated with the progression and development of HPV-related CESC. High levels of IGF2BP3led to disturbances in cell-cell and cell-ECM interactions. Therefore, targeting IGF2BP3 may interfere with viral infection by affecting the tight junction in HPV-related CESC.

Tight junctions preserve the integrity and stability of the epithelial barrier by regulating the paracellular flow of micro- and macromolecules between epithelial cells [52]. Previous researches demonstrated that tight junctions might play an import role in HPV-related diseases. E7 oncoprotein from human papillomavirus 16 has been shown to alter the expression levels of claudins and the closure of epithelial tight junctions, this process was shown to specifically include the reduced expression of claudins -1, -2 and -10, and an increase in the expresion of claudin-4 [53]. In addition, in HPV-positive tumor cells, E6-mediated the suppression of MAGI-1 function and contributed to HPV-induced malignancy by disrupting tight junction formation and by concurrently stimulating proliferation and inhibiting apoptosis [54, 55].

High expression levels of MARVELD3 is regarded as a potential prognostic biomarker for oral squamous cell carcinoma [56]. Furthermore, HPV infection results in the reduced expression of genes in tight junctions and can exert potential impact on HIV infection [57]. TJ proteins exert their functional role as integral proteins to form barriers in the physiology and disease biology of HPV infection, cervical intraepithelial neoplasia [CIN] and cervical cancer [58, 59]. In our data, the expression levels of most TJs were significantly higher in tumors than that in normal or para-carcinoma tissues, thus indicating potential causative role of tight junction abnormalities in CESC (Figure S2). This data concurs with previous studies involving cell line experiments and paired specimens from patients [60-63]. Furthermore, correlation analyses showed that IGF2BP3 was positively correlated with specific TJ proteins, including TJP1, TJP2, CLDN11, CLDN12, MARVELD2, MARVELD3, OCLN, MAGI2, LAMA3, LAMC2, ITGB4 and TJAP1 (Figure 4B, 4C). Collectively, these data indicated that IGF2BP3 might play a specific role in the formation of tight junction functions. In other studies, oncogenic HPV proteins were shown to trigger the mislocalization of ZO-2 TJ proteins from the cell borders to the cytoplasm and nucleus in MDCK cells [64]. Similarly, in another subcellular fractionation experiment, E6 oncoprotein was able to bind and induce the mislocalisation of Par3 protein in a PDZ-dependent manner without significant reduction in the protein levels of Par3 [65]. These results suggest that TJ proteins can also be expressed outside the tight junctions and play important functional roles in signaling, trafficking and the regulation of gene expression [66]. Therefore, the complex expression profile of TJ proteins in cells indicates that researchers should take the subcellular cytosolic or nuclear pool into consideration when investigating the causative role in pathogenesis of cancer.

It is important to consider that there are some limitations to our research. We should have collected our own clinical data to investigate the role of IGF2BP3 in the prognosis of patients of CESC. There were no More in-depth studies are needed to elucidate how HPV infection affects the expression of IGF2BP3. Furthermore, further research is needed to identify how IGF2BP3 plays a role in the HPV-mediated mechanisms that interfere with polarization machinery and signaling networks during viral replication and tumor development.

6. Conclusion

In this study, we investigated the association between IGF2BP3 and HPV status via several integrative analyses. We found that changes in IGF2BP3 influenced the cell-cell and cell-ECM interactions which may be responsible for CIN and an increased vulnerability to CESC. Furthermore, HPV infection may disrup the formation of junction barriers via IGF2BP3. High expression levels of IGF2BP3 in targets cell makes them more susceptible to HPV infection; consequently, IGF2BP3 represents a potential therapeutic target for anticancer therapy.

7. Declarations

7.1. Ethics Approval and Consent to Participate

Not applicable.

7.2. Consent for Publication

Not applicable.

7.3. Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

7.4. Competing Interests

The authors declare there is no conflict of interest.

7.5. Acknowledgements

This work was supported by Suzhou science and technology planning project [SLT201921, SZN2021011, SZM2021018, SKY2022091, SZM2022024].

References

1. Fan Z, Wu X, Li C, Chen H, Liu W, Zheng Y, et al. CAM-VT: A Weakly supervised cervical cancer nest image identification approach using conjugated attention mechanism and visual transformer. Comput Biol Med. 2023; 162: 107070.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71(3): 209-49.
3. Orji AF, Gimm G, Desai A, Parekh T. The Association of Cervical Cancer Screening with Disability Type among U.S. Women (25-64 years). Am J Prev Med. 2023.
4. Rideg O, Dergez T, Farkas K, Kovács K, Kálmán E, Tornóczky T, et al. High Prevalence of Non-Vaccinated Oncogenic Human Papillomavirus Genotypes in High-Grade Squamous Intraepithelial Lesions of the Cervix: Thought-Provoking Results of a Detailed HPV Genotype Analysis. Vaccines (Basel). 2022; 10(5).
5. Rodrigues LLS, Pilotto JH, Martinelli KG, Nicol AF, De Paula VS, Gheit T, et al. Diversity of Anal HPV and Non-HPV Sexually Transmitted Infections and Concordance with Genital Infections in HIV-Infected and HIV-Uninfected Women in the Tapajós Region, Amazon, Brazil. Viruses. 2023; 15(6).
6. Fischer S, Kuebler U, Abbruzzese E, Breymann C, Mernone L, Ehlert U, et al. Endogenous oestradiol and progesterone as predictors of oncogenic human papillomavirus (HPV) persistence. BMC Cancer. 2022; 22(1): 145.
7. Luo X, Donnelly CR, Gong W, Heath BR, Hao Y, Donnelly LA, et al. HPV16 drives cancer immune escape via NLRX1-mediated degradation of STING. J Clin Invest. 2020; 130(4): 1635-52.
8. Wang N, Zhu L, Wang L, Shen Z, Huang X. Identification of SHCBP1 as a potential biomarker involving diagnosis, prognosis, and tumor immune microenvironment across multiple cancers. Comput Struct Biotechnol J. 2022; 20: 3106-19.
9. Garcia-Martinez L, Zhang Y, Nakata Y, Chan HL, Morey L. Epigenetic mechanisms in breast cancer therapy and resistance. Nat Commun. 2021; 12(1): 1786.
10. Zhang Q, Zhao S, Su C, Han Q, Han Y, Tian X, et al. Construction of a Quantum-Dot-Based FRET Nanosensor through Direct Encoding of Streptavidin-Binding RNA Aptamers for N6-Methyladenosine Demethylase Detection. Anal Chem. 2023.
11. He L, Li H, Wu A, Peng Y, Shu G, Yin G. Functions of N6-methyladenosine and its role in cancer. Mol Cancer. 2019; 18(1): 176.
12. Kuang Y, Li R, Wang J, Xu S, Qiu Q, Lin S, et al. ALKBH5-mediated RNA m(6) A methylation regulates the migration, invasion and proliferation of rheumatoid fibroblast-like synoviocytes. Arthritis Rheumatol. 2023.
13. Han X, Wang L, Han Q. Advances in the role of m(6)A RNA modification in cancer metabolic reprogramming. Cell Biosci. 2020; 10: 117.
14. Yan C, Xiong J, Zhou Z, Li Q, Gao C, Zhang M, et al. A cleaved METTL3 potentiates the METTL3-WTAP interaction and breast cancer progression. Elife. 2023; 12.
15. Zhao NN, Liu YZ, Zhang L, Liu W, Zou X, Xu Q, et al. Construction of Multiple DNAzymes Driven by Single Base Elongation and Ligation for Single-Molecule Monitoring of FTO in Cancer Tissues. Anal Chem. 2023.
16. Zhang Z, Zhou K, Han L, Small A, Xue J, Huang H, et al. RNA m(6) A reader YTHDF2 facilitates precursor miR-126 maturation to promote acute myeloid leukemia progression. Genes Dis. 2023; 11(1): 382-96.
17. Zhuo R, Xu M, Wang X, Zhou B, Wu X, Leone V, et al. The regulatory role of N(6) -methyladenosine modification in the interaction between host and microbes. Wiley Interdiscip Rev RNA. 2022; 13(6): e1725.
18. Lang F, Singh RK, Pei Y, Zhang S, Sun K, Robertson ES. EBV epitranscriptome reprogramming by METTL14 is critical for viral-associated tumorigenesis. PLoS Pathog. 2019; 15(6): e1007796.
19. Xue M, Zhao BS, Zhang Z, Lu M, Harder O, Chen P, et al. Viral N(6)-methyladenosine upregulates replication and pathogenesis of human respiratory syncytial virus. Nat Commun. 2019; 10(1): 4595.
20. Courtney DG, Kennedy EM, Dumm RE, Bogerd HP, Tsai K, Heaton NS, et al. Epitranscriptomic Enhancement of Influenza A Virus Gene Expression and Replication. Cell Host Microbe. 2017; 22(3): 377-86.e5.
21. Wendt L, Pickin MJ, Bodmer BS, Reiche S, Fénéant L, Hölper JE, et al. N(6)-methyladenosine is required for efficient RNA synthesis of Ebola virus and other haemorrhagic fever viruses. Emerg Microbes Infect. 2023; 12(2): 2223732.
22. Yu R, Wei Y, He C, Zhou P, Yang H, Deng C, et al. Integrative Analyses of m6A Regulators Identify that METTL3 is Associated with HPV Status and Immunosuppressive Microenvironment in HPV-related Cancers. Int J Biol Sci. 2022; 18(9): 3874-87.
23. Condic M, Thiesler T, Staerk C, Klümper N, Ellinger J, Egger EK, et al. N6-methyladenosine RNA modification (m6A) is of prognostic value in HPV-dependent vulvar squamous cell carcinoma. BMC Cancer. 2022; 22(1): 943.
24. Zhao J, Lee EE, Kim J, Yang R, Chamseddin B, Ni C, et al. Transforming activity of an oncoprotein-encoding circular RNA from human papillomavirus. Nat Commun. 2019; 10(1): 2300.
25. Hu C, Liu T, Han C, Xuan Y, Jiang D, Sun Y, et al. HPV E6/E7 promotes aerobic glycolysis in cervical cancer by regulating IGF2BP2 to stabilize m(6)A-MYC expression. Int J Biol Sci. 2022; 18(2): 507-21.
26. Torres-Flores JM, Arias CF. Tight Junctions Go Viral! Viruses. 2015; 7(9): 5145-54.
27. Torres-Flores JM, Silva-Ayala D, Espinoza MA, López S, Arias CF. The tight junction protein JAM-A functions as coreceptor for rotavirus entry into MA104 cells. Virology. 2015; 475: 172-8.
28. Zhang J, Yang W, Roy S, Liu H, Roberts RM, Wang L, et al. Tight junction protein occludin is an internalization factor for SARS-CoV-2 infection and mediates virus cell-to-cell transmission. Proc Natl Acad Sci U S A. 2023; 120(17): e2218623120.

29. B. RD, G. CZ, S. C, T. K, C. AL, N. CS, et al. Integrated genomic and molecular characterization of cervical cancer. Nature. 2017; 543(7645): 378-84.
30. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15(12): 550.
31. Walter W, Sánchez-Cabo F, Ricote M. GOplot: an R package for visually combining expression data with functional analysis. Bioinformatics. 2015; 31(17): 2912-4.
32. Gallego C, Jaracz-Ros A, Laganà M, Mercier-Nomé F, Domenichini S, Fumagalli A, et al. Reprogramming of connexin landscape fosters fast gap junction intercellular communication in human papillomavirus-infected epithelia. Front Cell Infect Microbiol. 2023; 13: 1138232.
33. Glazier R, Salaita K. Supported lipid bilayer platforms to probe cell mechanobiology. Biochim Biophys Acta Biomembr. 2017; 1859(9 Pt A): 1465-82.
34. Lee KJ, Kim JY, Choi JH, Kim HG, Chung YC, Roh SH, et al. Inhibition of tumor invasion and metastasis by aqueous extract of the radix of Platycodon grandiflorum. Food Chem Toxicol. 2006; 44(11): 1890-6.
35. He J, Zhang N, Zhu Y, Jin R, Wu F. MSC spheroids-loaded collagen hydrogels simultaneously promote neuronal differentiation and suppress inflammatory reaction through PI3K-Akt signaling pathway. Biomaterials. 2021; 265: 120448.
36. Ganieva U, Schneiderman S, Bu P, Beaman K, Dambaeva S. IL-22 regulates endometrial regeneration by enhancing tight junctions and orchestrating extracellular matrix. Front Immunol. 2022; 13: 955576.
37. Wen Q, Li N, Xiao X, Lui WY, Chu DS, Wong CKC, et al. Actin nucleator Spire 1 is a regulator of ectoplasmic specialization in the testis. Cell Death Dis. 2018; 9(2): 208.
38. Liu T, Wei Q, Jin J, Luo Q, Liu Y, Yang Y, et al. The m6A reader YTHDF1 promotes ovarian cancer progression via augmenting EIF3C translation. Nucleic Acids Res. 2020; 48(7): 3816-31.
39. Lv D, Gimple RC, Zhong C, Wu Q, Yang K, Prager BC, et al. PDGF signaling inhibits mitophagy in glioblastoma stem cells through N(6)-methyladenosine. Dev Cell. 2022; 57(12): 1466-81.e6.
40. Shan TD, Han Y, Song MQ, Chen L. Mettl14-mediated m(6) A modification regulates the abnormal differentiation of small intestinal epithelial stem cells in diabetic state. J Cell Physiol. 2023.
41. Wang X, Chen C, Sun H, Mao K, Yao J, Zhang W, et al. m(6)A mRNA modification potentiates Th17 functions to inflame autoimmunity. Sci China Life Sci. 2023.
42. Jia Y, Yu X, Liu R, Shi L, Jin H, Yang D, et al. PRMT1 methylation of WTAP promotes multiple myeloma tumorigenesis by activating oxidative phosphorylation via m6A modification of NDUFS6. Cell Death Dis. 2023; 14(8): 512.
43. Xu Y, Ye S, Zhang N, Zheng S, Liu H, Zhou K, et al. The FTO/miR-181b-3p/ARL5B signaling pathway regulates cell migration and invasion in breast cancer. Cancer Commun (Lond). 2020; 40(10): 484-500.
44. Shriwas O, Priyadarshini M, Samal SK, Rath R, Panda S, Das Majumdar SK, et al. DDX3 modulates cisplatin resistance in OSCC through ALKBH5-mediated m(6)A-demethylation of FOXM1 and NANOG. Apoptosis. 2020; 25(3-4): 233-46.
45. Yuan J, Guan W, Li X, Wang F, Liu H, Xu G. RBM15-mediating MDR1 mRNA m(6)A methylation regulated by the TGF-β signaling pathway in paclitaxel-resistant ovarian cancer. Int J Oncol. 2023; 63(4).
46. Müller S, Glaß M, Singh AK, Haase J, Bley N, Fuchs T, et al. IGF2BP1 promotes SRF-dependent transcription in cancer in a m6A- and miRNA-dependent manner. Nucleic Acids Res. 2019; 47(1): 375-90.
47. Wang X, Hu Y, Li X, Zhu C, Chen F. YTHDC2-mediated m6A mRNA modification of Id3 suppresses cisplatin resistance in non-small cell lung cancer. J Thorac Dis. 2023; 15(3): 1247-57.
48. Klein S, Wuerdemann N, Demers I, Kopp C, Quantius J, Charpentier A, et al. Predicting HPV association using deep learning and regular H&E stains allows granular stratification of oropharyngeal cancer patients. NPJ Digit Med. 2023; 6(1): 152.
49. Li Y, Jiang YJ, Nie KH, Li J, Li YF, Huang CZ, et al. Rational design of genotyping nanodevice for HPV subtype distinction. Anal Chim Acta. 2023; 1276: 341651.
50. Tuominen H, Al-Samadi A, Salo T, Rautava J. Human myoma tissue-based extracellular matrix models for testing the effects of irradiation on the HPV positive cells. Virol J. 2020; 17(1): 87.
51. Wüstenhagen E, Boukhallouk F, Negwer I, Rajalingam K, Stubenrauch F, Florin L, et al. The Myb-related protein MYPOP is a novel intrinsic host restriction factor of oncogenic human papillomaviruses. Oncogene. 2018; 37(48): 6275-84.
52. Sharma D, Malik A, Guy CS, Karki R, Vogel P, Kanneganti TD. Pyrin Inflammasome Regulates Tight Junction Integrity to Restrict Colitis and Tumorigenesis. Gastroenterology. 2018; 154(4): 948-64.e8.
53. Uc PY, Miranda J, Raya-Sandino A, Alarcón L, Roldán ML, Ocadiz-Delgado R, et al. E7 oncoprotein from human papillomavirus 16 alters claudins expression and the sealing of epithelial tight junctions. Int J Oncol. 2020; 57(4): 905-24.
54. Kranjec C, Banks L. A systematic analysis of human papillomavirus (HPV) E6 PDZ substrates identifies MAGI-1 as a major target of HPV type 16 (HPV-16) and HPV-18 whose loss accompanies disruption of tight junctions. J Virol. 2011; 85(4): 1757-64.
55. Kranjec C, Massimi P, Banks L. Restoration of MAGI-1 expression in human papillomavirus-positive tumor cells induces cell growth arrest and apoptosis. J Virol. 2014; 88(13): 7155-69.
56. Huang K, Meng Y, Lu J, Xu L, Wang S, Wang H, et al. High expression of MARVELD3 as a potential prognostic biomarker for oral squamous cell carcinoma. Front Genet. 2022; 13: 1050402.
57. Britto AMA, Goes LR, Sivro A, Policarpo C, Meirelles Â R, Furtado Y, et al. HPV Induces Changes in Innate Immune and Adhesion Molecule Markers in Cervical Mucosa With Potential Impact on HIV Infection. Front Immunol. 2020; 11: 2078.

58. Berndt P, Winkler L, Cording J, Breitkreuz-Korff O, Rex A, Dithmer S, et al. Tight junction proteins at the blood-brain barrier: far more than claudin-5. Cell Mol Life Sci. 2019; 76(10): 1987-2002.

59. Chen B, Bu R, Xu X. Expression of Tight Junction Proteins Is Altered in Bladder Cancer. Anal Cell Pathol (Amst). 2020; 2020: 6341256.

60. Simon AG, Lyu SI, Laible M, Wöll S, Türeci Ö, Şahin U, et al. The tight junction protein claudin 6 is a potential target for patient-individualized treatment in esophageal and gastric adenocarcinoma and is associated with poor prognosis. J Transl Med. 2023; 21(1): 552.

61. Leinster DA, Colom B, Whiteford JR, Ennis DP, Lockley M, McNeish IA, et al. Endothelial cell junctional adhesion molecule C plays a key role in the development of tumors in a murine model of ovarian cancer. Faseb j. 2013; 27(10): 4244-53.

62. Caruso M, Fung KY, Moore J, Brierley GV, Cosgrove LJ, Thomas M, et al. Claudin-1 Expression Is Elevated in Colorectal Cancer Precursor Lesions Harboring the BRAF V600E Mutation. Transl Oncol. 2014; 7(4): 456-63.

63. Kimbung S, Kovács A, Bendahl PO, Malmström P, Fernö M, Hatschek T, et al. Claudin-2 is an independent negative prognostic factor in breast cancer and specifically predicts early liver recurrences. Mol Oncol. 2014; 8(1): 119-28.

64. Hernández-Monge J, Garay E, Raya-Sandino A, Vargas-Sierra O, Díaz-Chávez J, Popoca-Cuaya M, et al. Papillomavirus E6 oncoprotein up-regulates occludin and ZO-2 expression in ovariectomized mice epidermis. Exp Cell Res. 2013; 319(17): 2588-603.

65. Facciuto F, Bugnon Valdano M, Marziali F, Massimi P, Banks L, Cavatorta AL, et al. Human papillomavirus (HPV)-18 E6 oncoprotein interferes with the epithelial cell polarity Par3 protein. Mol Oncol. 2014; 8(3): 533-43.

66. Wu CJ, Mannan P, Lu M, Udey MC. Epithelial cell adhesion molecule (EpCAM) regulates claudin dynamics and tight junctions. J Biol Chem. 2013; 288(17): 12253-68.