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Chemical Biology
LETTERS

In silico analysis of the role of hsa-miR-155-5p in cervical cancer

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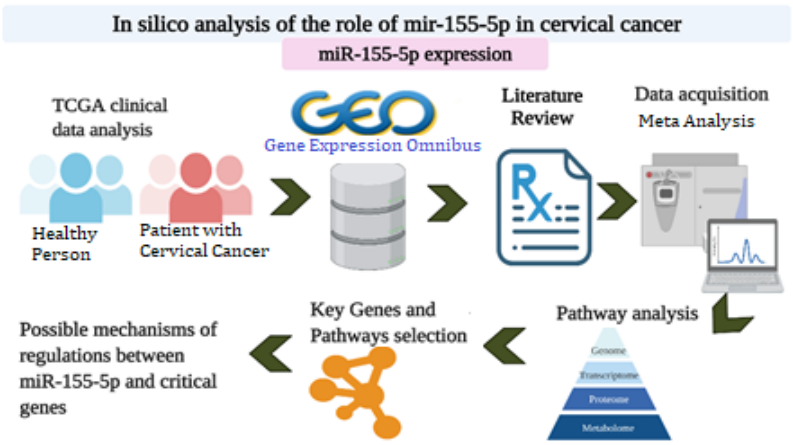
ABSTRACT



Numerous studies have established a critical role of micro-RNAs in the transcriptional regulation of multiple genes during cancer pathogenesis. Several micro-RNAs are associated with the poor prognosis and outcome of cervical cancer implicating their potential role in therapeutic intervention. The aim of this study was to determine the role of miR-155-5p in cervical cancer by performing a meta-analysis on its expression and identifying its molecular targets and pathways based on Gene Expression Omnibus (GEO) dataset, The Cancer Genome Atlas (TCGA) dataset, and literature review. Meta-analysis confirmed the upregulation of miR-155-5p expression in cervical cancer that significantly correlated with the numbers of tumour purity and histopathological grades. By using integrated bioinformatics approach, this study demonstrates that miR-155-5p could promote cervical cancer progression through targeting the expression of *Sp1, EGFR, UBR4* and *Pik3R1* genes. Importantly, these four genes play a crucial role in estrogen signaling pathway and choline metabolism. This study may provide future insights in revealing the mechanisms underlying pathogenesis of cervical cancer.

Keywords: *Cervical cancer, Meta-analysis, miR-155-5p, Gene ontology, TCGA dataset, Pathway enrichment*

INTRODUCTION

Cancer of the cervix is one of the most frequent malignancies affecting the female population with Human Papilloma Virus (HPV) infection as the major risk factor.[1] Cervical cancer ranks fourth in both incidence and mortality rates in women worldwide, with about 0.6 million cases and 0.3 million deaths annually.[2,3] There is a substantial decline in the incidence rates in developed countries, due to improvements in maternal health, wide-scale early screening and HPV vaccination.[4] However, due to ineffective screening as well as geographical and socio-economic disparities, the developing countries are the worst affected by this disease.[5]

In cervical cancer, there is a long pre-invasive window period that allows for early detection of the disease.[6] The early diagnosis of pre-malignant lesions is the key to reduce the increasing pre-mature mortality rates in low resource countries. Although, the conventional Pap smear test detects cervix cancer in pre-cancerous stages, and has been the major preventive tool so far, there is a need to develop other strategies that serve as effective screening alternatives to decelerate the mortality rates, particularly in the resource limited countries.

Micro-RNAs are a class of short non-coding RNAs that bind to the RNA-induced silencing complex (RISC) to silence its target genes through degradation or translation repression during both normal and pathological conditions.[7] Dysregulated micro-RNAs may either perform an oncogenic role or function as tumour suppressors depending on the function of their target genes. The role of microRNAs in the tumorigenesis of many cancers including lung cancer,[8] breast cancer,[9] ovarian cancer,[10] head and neck cancer[11] have been widely studied. Our study and other studies in the past[12-16] have delineated a significant role of miR-155-5p in the diagnosis and prognosis of cervical cancer.[13,14,15] High expression levels of miR-155-5p were observed in the pap smear of squamous intraepithelial cervical

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cancer patients compared to healthy individuals, suggesting their significance as predictive biomarker.[16] A study revealed that miR-155-5p promotes metastasis in cervical carcinoma through targeting TP53INP1.[15] Another study by Park, S demonstrated the diagnostic significance of mir-155-5p in predicting the risk of HPV infection in cervical cancer patients.[13] MiR-155-5p was also studied to stimulate autophagy in cervical cancer cells by suppressing the PDK1/mTOR signaling.[17] In our previous study, we demonstrated for the first time the expression profile of miR-155-5p in urine for their potential as effective non-invasive biomarkers in the early diagnosis of cervical cancer.[16] This study aimed to analyse the functions of miR-155-5p in cervical cancer by performing comprehensive analysis on its expression to identify its putative molecular targets and pathways by utilizing different bioinformatics algorithms based on datasets from The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), and literature review. We have also analysed the target genes of miR-155-5p for enrichment annotation to identify functional clusters. Finally, we have constructed networks to predict the possible pathways where the miR-155-5p could be involved, providing a resource for further understanding of the molecular mechanisms, and develop early diagnostic and treatment strategies in cervical cancer.

RESULTS

Clinical relevance of miR-155-5p

A comparative representation of the miR-155-5p expression data in cervical cancer (CC) and healthy samples is provided in the TCGA. As shown in Figure 1, a 9.24 fold upregulation in miR-155-5p expression normalised to healthy controls was observed in CC patients implying its clinical significance. MiR-155-5p was observed to be significantly associated with the tumour status (P = 0.0006) and histological type (P= 3.29E-09). However, there was no significant correlation with the number of lymph nodes, race, pathology N stage, number of years of birth, radiotherapy, or overall survival (Table 1).

Meta-analysis of miR-155-5p expression based on TCGA/GEO data

A total of 4320 gene expression datasets are available on the Gene Expression Omnibus (GEO) repository. After careful screening, four datasets: GSE19611, GSE30656, GSE105409 and GSE86100 were selected out of which three met the eligibility criteria and were enrolled in the meta-analysis. A forest plot of miR-155-5p expression in CC and healthy controls is shown in Figure.2a and b. Meta-analysis showed a significant heterogeneity (I^2 = 97%) using random effects model and a significant pooled standard mean difference (SMD) of 3.02 (95% CI [-0.59-6.63], P < 0.00001). These data indicate an upregulated expression of miR-155-5p in cervical cancer (Figure. 2a). A funnel plot of miR-155-5p expression (Figure 2c) reveals that no significant publication bias is detected by Egger's test.

Meta-analysis of miR-155-5p expression based on literature search

A total of four eligible studies[13-16] were selected for meta-analysis. Consistent with the results of the TCGA/GEO meta-analysis, a common pattern of upregulated miR-155-5p expression in cervical cancer was observed across the included studies (Table 2).

Table 1: Correlations between miR-155-5p expression and clinical outcomes

Item	Method	Correlation	P Value	Q Value
Number of lymph nodes	Spearman Correlation	0.1072	0.1788	0.95
Tumour status	Wilcox Test	0.6299	0.0006801	0.0902
Race	Kruskal-Wallis Test	0.3291	0.5004	0.765
Clinical stage	Kruskal-Wallis Test	0.7893	0.113	0.586
Years to birth	Wilcox Test	0.0342	0.5508	0.906
Histological type	Kruskal–Wallis Test	0.6543	3.29E-09	1.37E-07
Ethnicity	Wilcox Test	0.52	0.7529	0.964
Radiation therapy	Wilcox Test	0.5405	0.3855	0.887
Pathology N stage	Wilcox Test	0.5321	0.999	0.456
Overall survival	Spearman correlation	-0.0074	0.9811	1

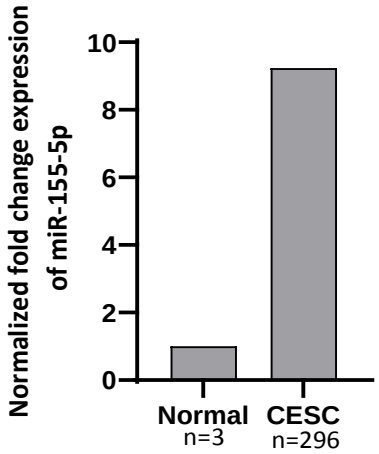


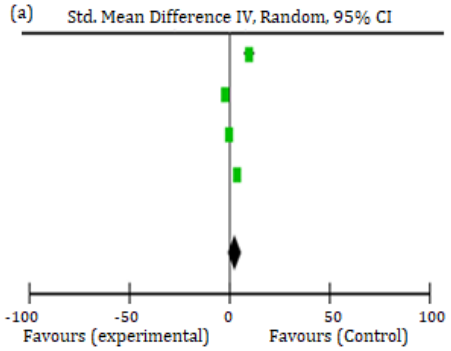
Figure 1; Expression of miR-155-5p in cervical cancer from TCGA

Bioinformatics analyses of miR-155-5p

Screening of miR-155-5p target genes

Differentially expressed genes (DEGs) between CC and healthy controls in CESC dataset were sorted out using GEPIA software with the criterion of log|FC| > 1 and FDR < 0.05. Out of 6061 DEGs, 3195 were found to be upregulated and 2866 were downregulated. Alternatively, miR-155-5p target genes were predicted based on 10 databases in miRWalk. 15110 genes were consistently identified as miR-155-5p targets in at least five databases (Figure. 3a). After merging DEGs and the predicted target genes, 1530 candidate genes were identified as potential miR-155-5p targets by Venn diagram analysis (Figure 3b).

	Experimental			Control				Std. Mean Difference IV
Study of Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	Random 95%CI
GSE 105409	36.23	3.67	30	5.42	0.45	16	23.7%	10.12 (7.88, 12.37)
GSE 19611	3.66	1.27	6	6	1.27	6	25.1%	-1.70 (-3.10, -0.30)
GSE 86100	0.64	0.4	11	0.47	0.72	23	25.8%	0.26 (-0.46,0.98)
TCGA 2020	8.01	0.707	296	5.28	0.27	3	25.4%	3.86 [2.68,5.04]
Total (95% CI)			343			48	100.0%	1.32 [0.77,1.87]
Heterogeneity: ChI2 = 102.92, df = 3 (P < 0.00001); I2 = 97 %) Test for overall effect; Z = 1.64 (P = 0.10)								



	Experimental			Control				Std. Mean Difference IV
Study of Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	Fixed 95%CI
GSE 105409	36.23	3.67	30	5.42	0.45	16	23.7%	10.12 (7.88, 12.37)
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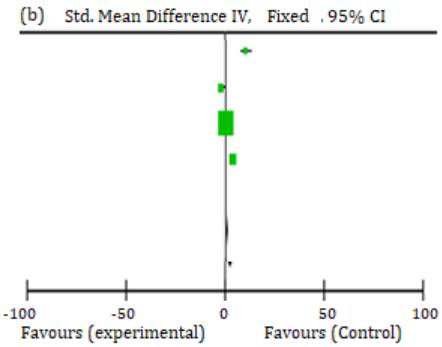

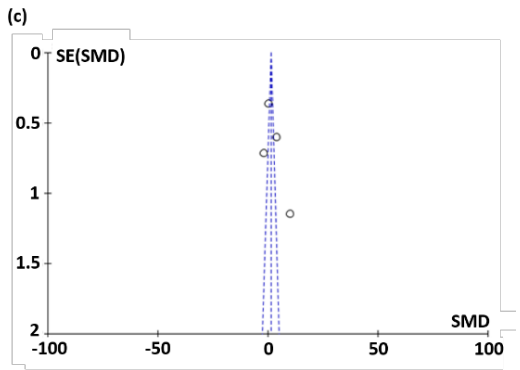

Figure 2. Meta-analysis of miR-155-5p between healthy control and cancerous cervical tissue based on TCGA and GEO. Forest plot of SMD (a)Random effect Model (b) Fixed effect Model. The expression of miR-155-5p is significantly higher in cervical cancer tissue; c Funnel plot for four studies that are marked as circles. No significant publication bias is detected (P<0.00001)

Table 2: Overview of the four studies selected in the literature Review

Author	Year	Country	Cervix Cancer(n)	Normal (n)	Result	Detection Methods
Ning Li et.al	2019	China	24	24	Upregulated	qRT-PCR
Lao G et.al	2014	China	20	20	Upregulated	qRT-PCR
Park et. al	2017	China	52	50	Upregulated	qRT-PCR
Aftab et.al	2021	India	50	50	Upregulated	qRT-PCR

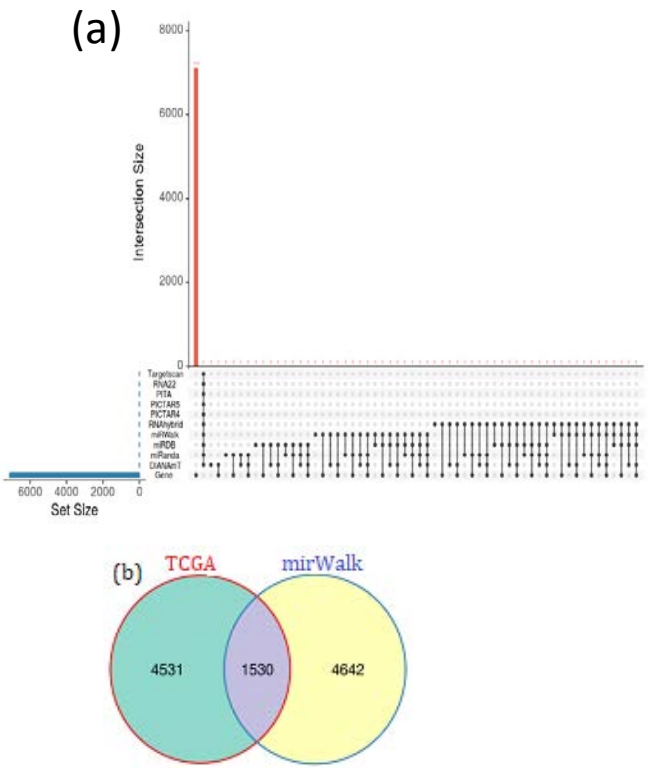

Figure 3 (a-b); Predication of miR-155-5p target genes and candidate genes screening. (a) The number of overlapped genes across 12 databases; 15110 target genes which overlapped at least five databases are obtained. (b) Venn plot for the integration between DEGs and predicted target genes of miR-155-5p

Gene ontology enrichment analysis of predicted miR-155-5p targets Ontology (GO) analysis of the 1530 genes (Figure. 4). Using the criterion of $P < 0.001$, it was observed that the cellular component (CeC) was significantly enriched in the extracellular space.

The DAVID database was used for Gene e, nucleus, external side of plasma membrane, cell-cell adherens junction, integral component of the plasma membrane, cell-cell junction, focal adhesion and lateral plasma membrane. In terms of biological processes (BP), the target genes were mainly involved in protein phosphorylation, regulation of cell migration, lipopolysaccharide-mediated signaling pathway, cellular response to fibroblast growth factor stimulus, positive regulation of peptidyl-tyrosine phosphorylation, cellular response to organic cyclic compound, cell maturation, positive regulation of NF-kappa B transcription factor activity, angiogenesis, negative and positive regulation of transcription from RNA polymerase II

promoter and regulation of the apoptotic process. With regards to molecular function (MF), these genes were enriched in cytokine activity, damaged DNA binding, protein binding, growth factor activity, G-protein beta/gamma-subunit complex binding, and TFIID-class transcription factor binding.

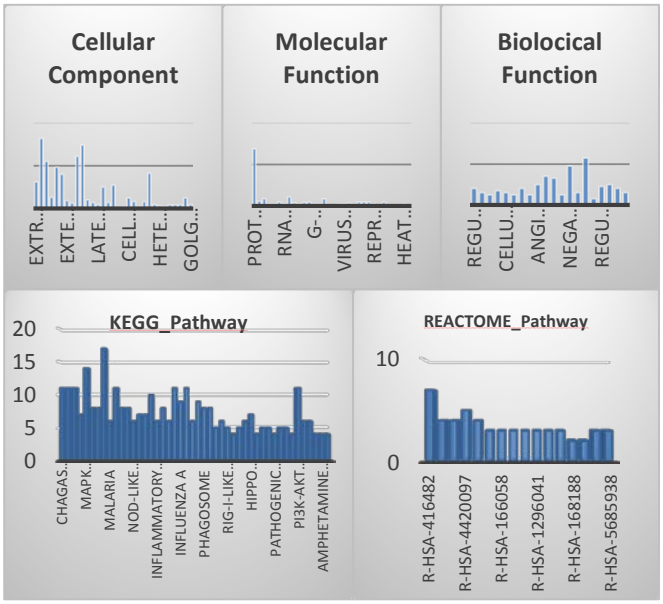

Figure 4; The top 20 items of cellular component (CC), molecular function (MF), and biological process (BP) pathways in Gene Ontology (GO) and pathway enrichment analysis for candidate target genes of miR-155-5p in cervical cancer. Values are expressed as −log10 (P-value)

Protein–protein interaction network analysis

Protein interactions among the identified miR-155-5p target genes were analyzed by the STRING online database. In the original network a total of 1368 nodes and 7131 edges were identified (Figure.5a). By extracting the nodes/key genes with degree and betweenness values higher than average, a subnetwork containing 38 nodes and 269 edges was constructed (Figure 5b; Table 3). Twelve topological algorithms were applied and the top 20 genes of each method for the subnetwork of PPI were extracted. The selected genes that appeared at least twice were conserved as hub genes (Table S1).

Pathway enrichment and crosstalk analysis

Pathway enrichment analysis was also performed by the DAVID database. The results indicate that the NF kappa B signaling, negative regulation of apoptosis, and regulation of cell migration pathways were significantly enriched. 43 pathways that contained more than two genes fulfilled the crosstalk analysis criteria and were selected to construct the pathway cross talk network (Figure.5c). The thickness of the edges indicates measurements of the average value of OC and JC. A major cluster was identified from the initial network with 33 nodes and 361 edges using MCODE (Figure.5d).

Comprehensive analysis of gene-pathway

After mapping the hub genes into the sub-network of pathways guided by KEGG, a potential gene-pathway network including 34 essential pathways and 16 hub genes was constructed (Figure. 5e). This network shows that PIK3R1 and EGFR were major participants in most of the pathways. Furthermore, estrogen signaling pathway, MAPK signaling pathway, pathways in cancer, ras signaling pathway and PI3K-Akt signaling pathway were the top ranked pathways according to the genes involved. To screen the major factors (including genes and pathways) in the gene-pathway network, the nodes with a degree value > average were selected (Figure. 5f). It was found that eight genes (CREB1, FOS, FOXO3, PIK3R1, EGFR, Sp1, UBR4 and RELA) with ten pathways (Choline metabolism in cancer, viral carcinogenesis, cAMP signaling pathways, B cell receptor signaling pathway, estrogen signaling pathways, HTLV-I infection, HIF signaling pathways, T cell receptor signaling pathways, Toll-like receptor signaling pathway, insuli resistance, and pathways in cancer) were predominantly involved.

Identification of key genes and pathways

The expression of the critical genes was investigated by meta-analysis of seven studies in Oncomine (Figure 6a). The results demonstrated that all the key genes were upregulated in cervical cancer, out of which expression of three genes (Sp1, EGFR and UBR4) were statistically significant. However, PIK3R1 expression was not determined to be a statistically significant target. We merged the pathways that each essential gene was a part of to figure out the most important pathways (Figure 6b). Two pathways, choline metabolism in cancer and estrogen signaling pathway were subsequently identified as significant.

Location and characteristic of the binding site

As presented in Figure. 7, all the binding sites for miR-155-5p were located in the 3′UTR of Sp1, EGFR, PI3KR1, and UBR4. A further inspection of the sequence of binding sites revealed that adenine (A) and uracil (U) constituted the major portion of the sequence.

DISCUSSION

The overexpression of miR-155-5p is associated with an increased risk of cervical cancer in HPV E6 and E7 positive cases. According to our previous study, miR-155-5p was upregulated in 150 paired samples of cervical tissue, cervical scrape, serum and urine of pre-cancer and cervical cancer cases[16]. In this study, we further utilised the TCGA-GEO database to affirm that miR-155-5p is significantly upregulated in cervical cancer and its expression is highly correlated with the histological type and tumour status. By using bioinformatics analyses we accounted that miR-155-5p plays a critical role in the progression of cervical cancer by interacting with four key genes *Sp1*, *EGFR*, *PIK3R1* and *UBR4*.

Specificity protein 1 (Sp1) transcription factor regulates the expression of multiple genes associated with cancer progression including the Sp1 gene itself. Sp1 participates in HPV16 mediated cervical cancer development by regulating the Wnt/β-catenin pathway[18]. Overexpression of Sp1 significantly decreased the G2/M arrest in cervical cancer cells[18]. Sp1 binding sites have been identified in the enhancer/promoter regions of several genes associated with cell growth and division. Interactions of Sp1 with proteins regulating cell cycle and tumour formation have been reported, suggesting a possible

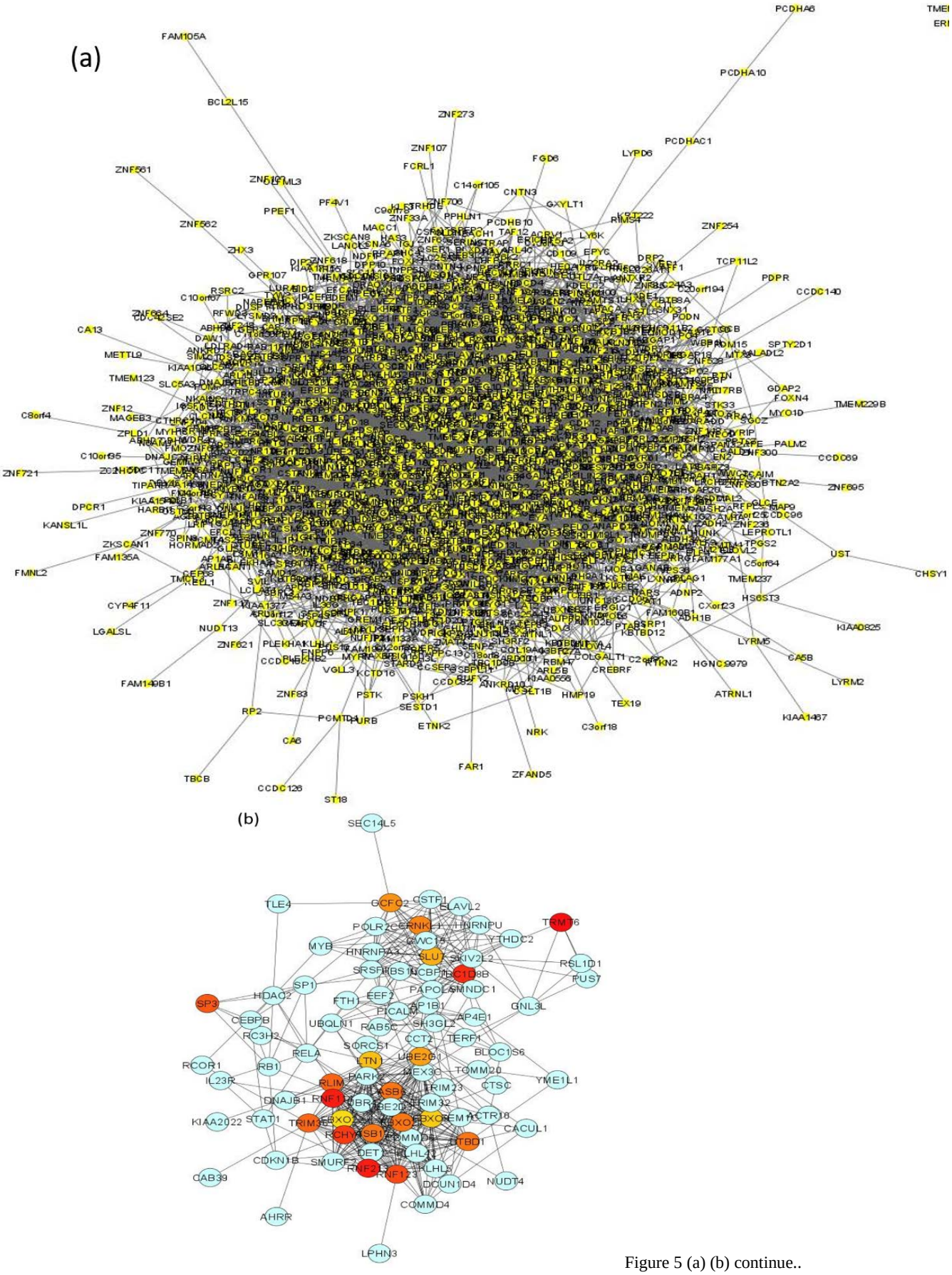

Figure 5 (a) (b) continue..

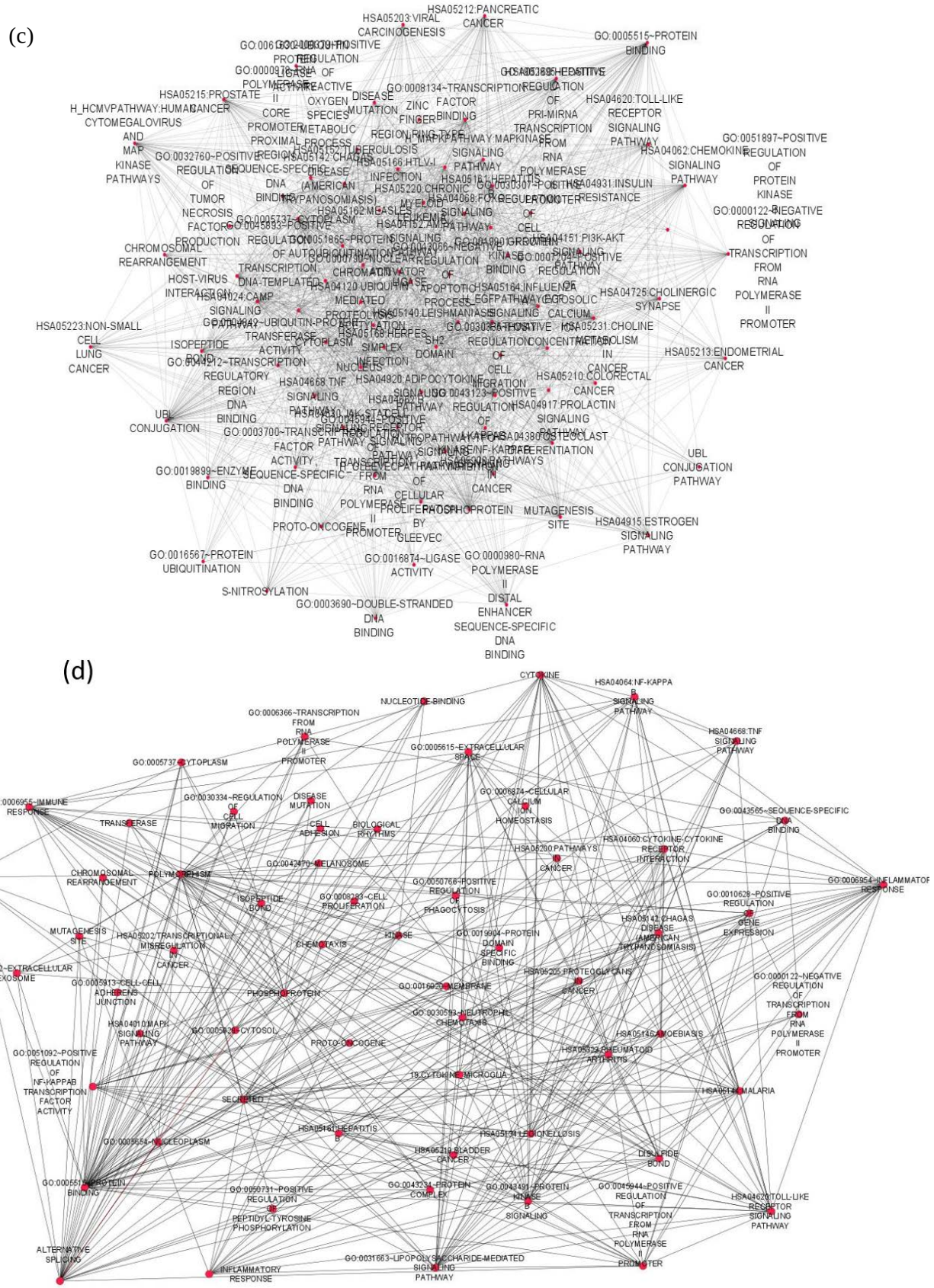

Figure 5 (c) (d) continue..

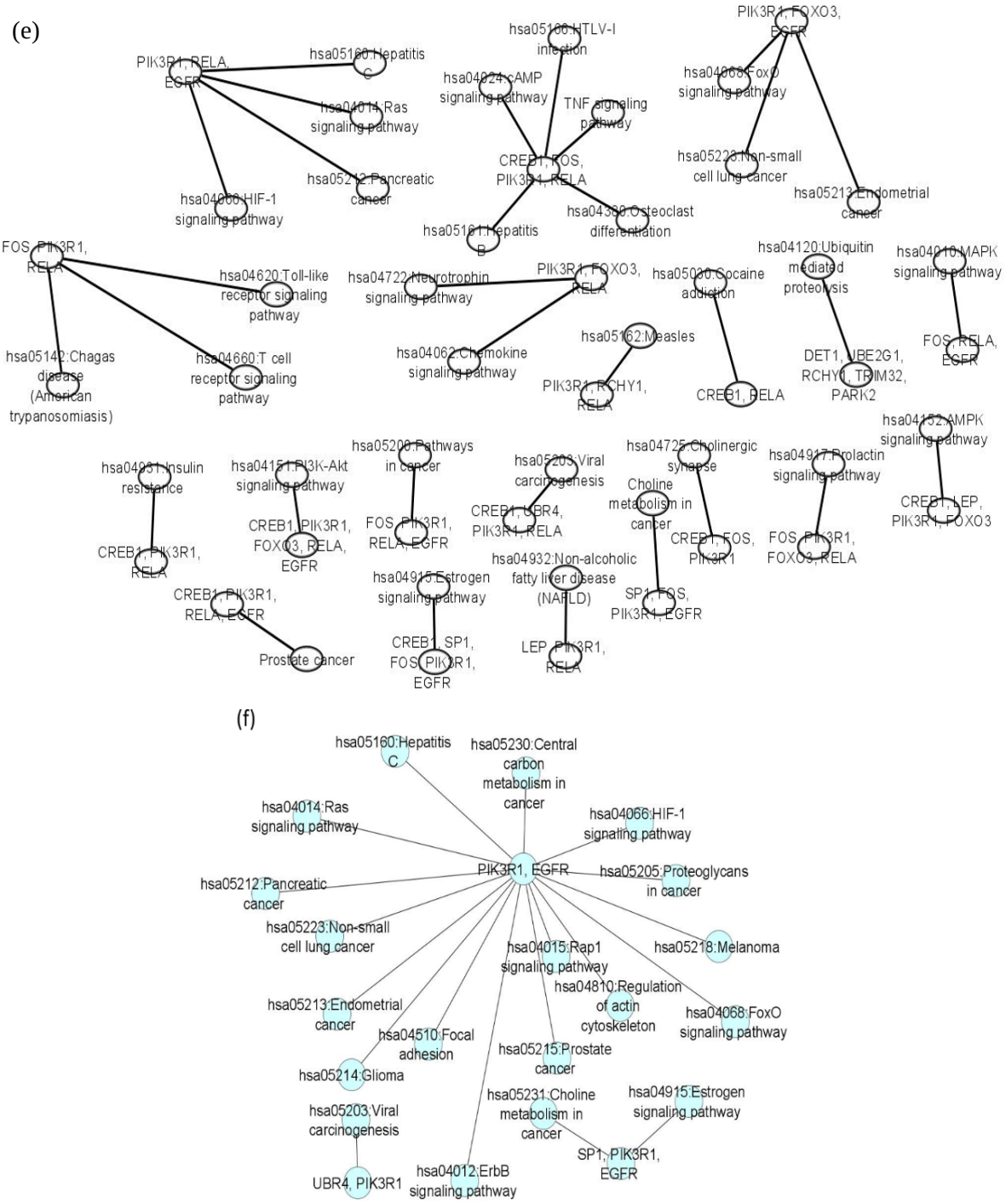

Figure 5. Protein–protein interaction (PPI) analysis a) PPI network of candidate genes; b) Subnetwork of PPI for main nodes extracted according to degree and betweenness being higher than average; c) Pathway crosstalk analysis of hub genes. The thickness of lines between nodes are represented by the average value of Jaccard coefficient (JC) and overlapping coefficient (OC); d) Subnetwork of pathway crosstalk extracted by MCODE; e) Comprehensive gene-pathway network constructed by mapping the hub genes to the subnetwork. f) Subnetwork of gene-pathway collected according to the criteria that node's degree > average

misregulation of genes with transcriptional sites for Sp1 in cervical cancer.[18,19]

EGFR is overexpressed in a wide variety of solid tumours.[20] Overexpression of EGFR in cervical cancer has been reported to be anywhere from 6% to 90% of cases[21]. The intensity of EGFR expression is elevated in higher grades of CIN, and is also reported to be significantly associated with HPV infection[21]. In addition, the oncogenic effect of HPV could be triggered by the mitogenic signaling mediated by the EGFR channel through the activation of Akt-1 and cyclin D1.[22-25]

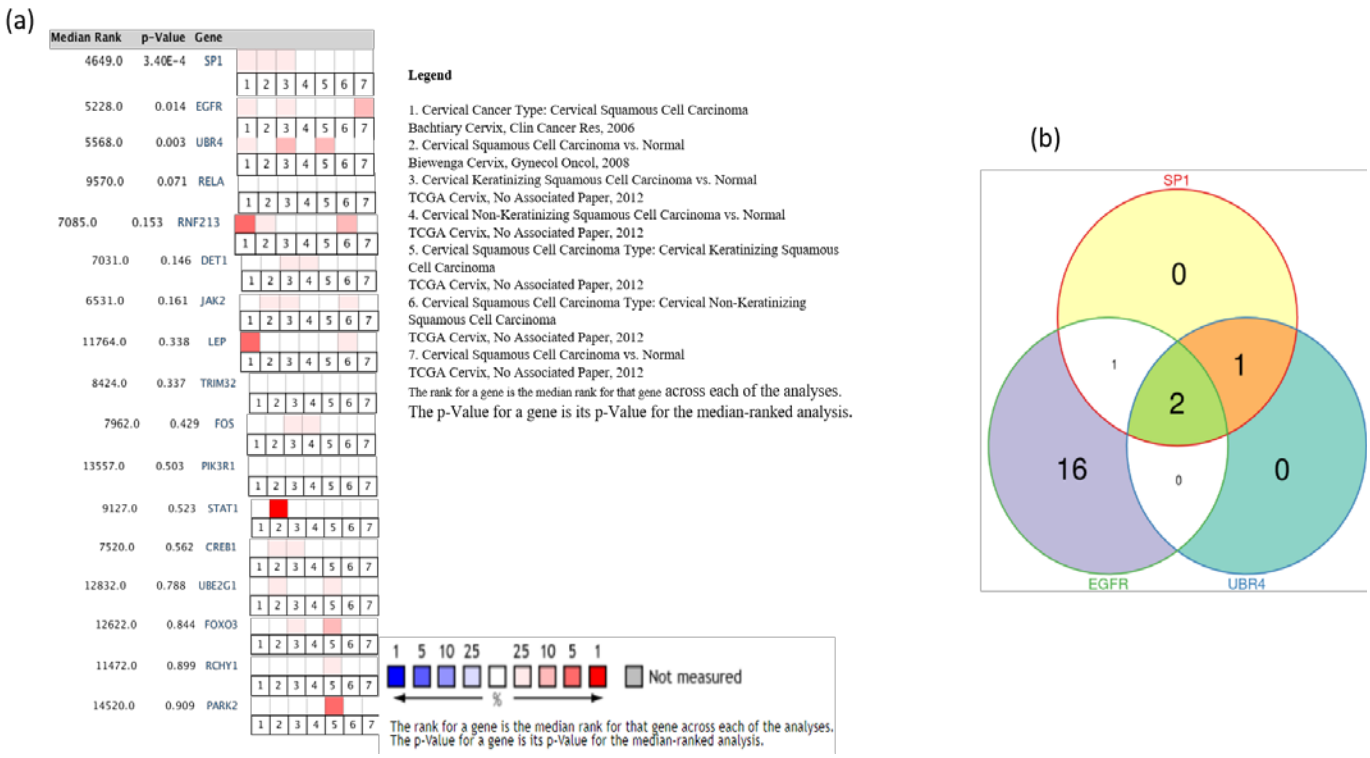

Figure 6 (a) The expression of main genes of cervical cancer across seven studies (b) Venn plot for the interaction between key genes and their Pathways

```
dataset: 1
target: PIK3R1        length: 6975
miRNA : hsa-mir-155    length: 65
mfe: -52.4 kcal/mol    p-value: 1.000000e+00
position  304

target 5' G       CAAGU       AA       A  C     GAGA
CUGU     CGCAGAGGAAGU    G        UU     CACAC
A 3'
           UGUUGA      UGCUGA      AGG AG CAGU     GGA
GGCA             GGA CCCUGUC   CGGU      CAUUGAUGG
           ACAAUU      ACGAUU      UCC UC GUCA     CCU
CCGU             UUU GGGAUAG   GCUA      GUAAUUGUC
miRNA  3' G                    AUACA       A     A
U               G        U      AUC              5'

dataset: 1
target: Sp1     length: 2214
miRNA : hsa-mir-155     length: 65
mfe: -55.7 kcal/mol    p-value: 1.000000e+00
position  37

target 5' G          AA    UGAAAA           GGCAAUAA
UGGUAAUGGUGGU  UG     U     A  CUCGAAG      GC      G
3'
           CUGU GGUG   AAU       AGGAGUUGGU
UGGGGGCAA              GG   CCUU UCAC GG         UAGCA
ACAG
           GACA UUAC   UUA       UCCUCAGUCA
ACCUCCGUU              UU   GGGA AGUG CU         AUCGU
UGUC
miRNA  3'       A     GA    UACA
UG     U        A            AAU       5'

dataset: 1
```

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miRNA : hsa-mir-155  length: 65
mfe: -53.1 kcal/mol  p-value: 1.000000e+00
position  6890

target 5'         A      CAG                    U      C
U        G UCUAUAAU    C       A 3'
                    GCUGA    AUGU GGAGUU    UGG GGUAA
GACC CCUA CA G          GCA   AACAG
                    CGAUU    UACA CCUCAG    ACC CCGUU
UUGG GGAU GU C          CGU   UUGUC
miRNA  3' GACAAUUA      A       U       UCA    U      U
A  G UAAU         AA        5'


dataset: 1
target: EGFR  length: 3633
miRNA : hsa-mir-155  length: 65
mfe: -54.2 kcal/mol   p-value: 1.000000e+00
position  2571

target 5' G   GCCAAAC           C  AA
AAAGAAUACCAUG    AA           UG         AG    A
GAAUCAAUUUUAC     A 3'
           CUG        UGCUGG GUG GG   GAG
CAG  GGAGGCAAAG   C CUAUCA   UGG UGGCAUUG
ACAG
           GAC        ACGAUU UAC UC   CUC
GUC   CCUCCGUUUU   G GAUAGU   GCU AUCGUAAU
UGUC
miRNA  3'      AAUU          A   A        A
AA           UG G            A
5'
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Figure 7 (a-d); Coding sequence and binding site of target genes and miRNA

UBR4 has been previously reported to bind to E7s from HPV16, 6b, and 11, and to bovine papillomavirus (BPV).[26] E7 as well suggesting that the interaction of UBR4 with E7 is highly conserved. The observation that UBR4 is bound to all HPV E7s, including those not associated with cancer indicates that this interaction may also have a key function in virus replication. Furthermore, ubiquitin ligase KCMF1 in complex with UBR4 suggests that the E7–UBR4 interaction may be an important determinant in the recruitment of additional cellular factors to complexes containing E7s.[27] For HPV16 E7, UBR4 binding appears to contribute to cellular transformation and anchorage-independent growth.[28]

Comprehensive gene pathway analysis revealed PI3KR1 gene as amongst the top targes of mir-155-5p. Inhibition of PI3K signaling is an effective treatment strategy in several types of cancer, including cervical cancer.[29]

The meta-analyses demonstrated that all the four gene targets of miR-155-5p were particularly involved in two key pathways: choline metabolism in cancer and estrogen signaling pathways. Aberrant choline metabolism is new metabolic hallmark reflecting the equal interactions between oncogenic signaling and cellular metabolism[30,.31] Molecular causes of abnormal choline metabolism have been investigated by determining enzymatic changes that result in an increased phosphocholine and total choline (tCho) levels, and in some cancers, accompanied by a relative decrease of glycerophosphocholine[32]. Enzymes mediating the abnormal choline metabolism are being explored as targets for cancer therapy. Choline kinase-α is phosphorylated by c-Src and was found to form a complex with EGFR that regulates cell proliferation and tumorigenesis.[33,34]

Several studies report the involvement of estrogen signaling in regulating cell cycle during carcinogenesis.[35] Others authors also have suggested that estrogen signaling pathway dysregulation in combination with HPV infection deteriorates disease progression.[36,37] Estrogen-receptor complex signaling is vast, appears to be cell type specific, and can interact with various other pathways including ras, Src and PI3 kinases, EGFR, AP-1, STATs, ATF-2/c-Jun, Sp1, NF-κB, and CREB[38]. The Lambert laboratory studies have demonstrated that the expression of the estrogen receptor α, E7, E6 and E5 expression is primarily linked to the development of HPV-related cervical dysplasia and cervical cancer.[39]

Binding site analysis interestingly revealed that the miR-155-5p binding sites were located in 3'UTR of Sp1, EGFR, PI3KR1, and UBR4 mRNAs. According to some studies one of the mechanisms of miRNA mediated gene upregulation involves direct binding of miRNA to 5'UTR of mRNA followed by enhanced translation by alleviating their TOP-mediated translational repression.[40]

MiRNAs can compete with AU-rich element- ARE-mediated mRNA decay (AMD). The AMD regulates the concentration of a class of mRNAs that contain AU-rich sequences within their 3′UTRs. ARE-binding proteins (ABPs) recruit the cytoplasmic mRNA degradation machinery to the target mRNAs leading to their 3′-to-5′ degradation.[41,42] It is through the degradation enzymes recruitment that the Tristetraprolin (TTP) protein family functions as a molecular link between ARE-containing mRNAs and the mRNA decay machinery. It is remarkably indicated that some miRNA-mediated regulation pathways may also have interactions with ARE-mediated pathways, as they share common binding sites in the mRNA 3′UTRs and have some common key players such as HuR, AGO2, CCR4, GW182, and decapping enzymes.[41,43] MicroRNAs therefore, can abrogate AMD by preventing ABPs associations leading to increased mRNA stability. The mechanisms of upregulation for cellular state or the binding site in 5′UTR may have a lesser possibility as none of the binding sites are located in the 5′UTR in our results, and cells are considered active in cancer tissue. AREs are found in the 3′UTR of mRNAs that code for proto-oncogenes, nuclear transcription factors, and cytokines. Our results reveal that A and U were the major constituents of the binding sequences[44]. It has been verified previously that TTP has interactions with EGFR, Sp1, PIK3R1, and UBR4. Furthermore, EGFR mRNA also contains some AREs, which indicates the possibility of EGFR binding to HuR.[45]

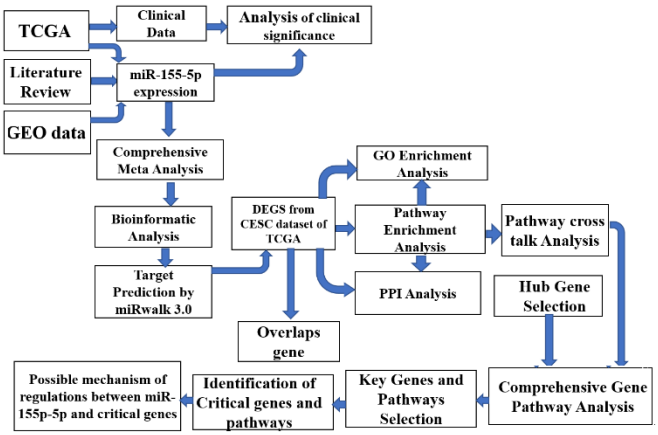

Figure 8: Work flow of the analysis of clinical significance and comprehensive analysis for miR-155-5p in cervical cancer.

METHODS

The flowchart representation of this study is illustrated in Figure.8. The study has been designed according to the guidelines of MIAME.[46] The clinical significance of miR-155-5p was assessed using information of cervical squamous cell carcinoma (CESC) patients from the TCGA database. The expression data of miR-155-5p was synthesized by meta-analysis of gene expression microarray datasets from the TCGA, GEO, and research literature. Differentially expressed genes (DEGs) in CESC patients from the TCGA were identified while 10 databases were screened to predict the target genes of miR-155-5p. Genes regulated in both groups were then further explored by bioinformatic analyses for functional annotation.

Correlation of miR-155-5p expression and clinical parameters

The clinical significance of miR-155-5p in cervical cancer was CESC-TP.merged_data.txt (http://gdac.broadinstitute.org/runs/stddata__2016_01_28/data/CESC/20160128/) which was constructed from the TCGA-CESC dataset that included 296 cancer samples and three normal

samples. The correlations between miR-155-5p expression and clinico-pathological parameters including metastatic lymph nodes, tumour purity, race, pathology N stage, years to birth, histological type, ethnicity, radiation therapy, and overall survival were analysed.

Meta-analysis of miR-155-5p expression based on literature and GEO/TCGA database

An organised search for literature based on miR-155-5p expression was performed from 4 electronic databases: PubMed, Web of Science, EMBASE and Cochrane Library. This was further supplemented by a manual screening of the references cited in the selected articles.

For retrieval of miR-155-5p expression data from the GEO/TCGA database, the following key terms were used: microRNA or miRNA or noncoding miRNA or cervix or cervical or cancer or carcinoma or tumour or neoplasia or malignancy or malignant.

For statistical analysis, the expression data were adjusted to normal distribution to reduce variation and, Log2 scale transformation was applied. The meta-analysis was conducted by Review Manager 5.4. The standardized mean difference (SMD) was pooled using the random effects model, and heterogeneity between studies was assessed using I^2tests where $P < 0.05$ or $I^2 >$ 50% was considered as significantly heterogenous. For $P > 0.05$, a fixed-effect model was used. Funnel plot with Egger's test was utilized to estimate the publication bias. $P < 0.1$ was considered to be significant asymmetry for the funnel plot. To perceive the strength of the pool results, sensitivity analysis was performed by different analysis model. In addition, to further evaluate the impact of individual studies on the overall effect estimates, influence analysis was performed.

Identification of candidate genes for miR-155-5p

The CESC data were analysed by using the HCMDB (Human Cancer Metastasis Database) to identify the differentially expressed genes (DEGs). We determined the significance of the difference in gene expression as Log2 |fold change (FC) |> 1 and False Discovery Rate (FDR) < 0.05. Furthermore, the miR-155-5p targeted genes were predicted by 10 established databases Microt4, miRWalk, mir-bridge, miRanda, miRDB, miRMap, Pictar2, PITA, miRNAMap, RNAhybrid, RNA22, and Targetscan using miRWALK version 3. To increase the prediction accuracy, the genes that were overlapping and common in at least five databases were selected. Finally, the overlap genes between DEGs and predicted genes were analyzed by upsetR and Venn Plot.

Functional enrichment analysis

To investigate the potential function of the predicted miR-155-5p target genes from the overlapping list, we performed Gene Ontology(GO;http://www.geneontology.org/) and pathway enrichment analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/). The top 20 significantly enriched biological items for cellular component (CeC), biological process (BP), and molecular functions (MF) were identified and plotted. A *P-value* < 0.01was considered as statistically significant.

Protein-protein interaction (PPI) network analysis

To construct the protein-protein interaction network between the predicted miR-155-5p target genes from the area of overlap, we used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING)[47]as PPI database. To acquire accurate results, the interactions with a combined score of < 0.7, and with a value of the degree and betweenness < average were excluded. A cytoscape plugin cytoHubba was utilised to predict and explore the important nodes or key genes that included 12 topological algorithms: Degree, Edge Percolated Component (EPC), Maximum Neighborhood Component (MNC), Density of Maximum Neighborhood Component (DMNC), Maximal Clique Centrality (MCC), and centralities based on shortest paths such as Bottleneck (BN), EcCentricity, Closeness, Radiality, Betweenness, Clustering Coefficient, and Stress. The top 20 predicted genes in each topological algorithm were extracted and the duplication of each gene was also calculated. The genes with < 2 in repetitiveness were excluded to ensure that the identified key genes were closely linked to cervical cancer while the rest were considered hub genes.

Crosstalk analysis among significantly enriched pathways

For pathway enrichment analysis, the predicted targets were mapped using the Kyoto Gene and Genome Encyclopedia (KEGG) database by DAVID. Significant pathways were considered as $P < 0.05$. The obtained pathways were further investigated for crosstalk analysis to explore their interactions. Two pathways were considered to crosstalk if they shared a proportion of miR-155-5p target genes. To computationally indicate the overlap of a pair of pathways, JC (Jaccard coefficient) = $| (A \cap B) / (A \cup B) |$ and OC (overlapping coefficient) = $(|A \cap B|) / (\min(|A|, |B|))$ was adopted, where A and B denote the number of candidate genes contained in the two pathways.. Any of the pathways that contained less than three genes were excluded as they may not be biologically meaningful. Further, pathway pairs sharing less than two genes were eliminated to ensure statistical significance. On the pathway crosstalk network, the Cytoscape plug in Molecular Complex Detection (MCODE) was applied to screen the hub genes with a score >4.

Comprehensive gene-pathway analysis

The hub genes were mapped into the subnetwork of crosstalk to further explore the mechanism by KEGG. To screen the key genes and pathways, the nodes with a degree value > average were collected for constituting a subnetwork.

Identification of key genes and pathways

For further validation, we also evaluated the expression of key miR-155-5p target genes by meta-analysis of CC and healthy samples using Oncomine. p value < 0.05 was considered as a significant difference. Moreover, influence analysis was also conducted to access the pool estimates. The pathways that all of the key genes participated in, were determined as crucial pathways.

Possible mechanisms of regulation between miR-155-5p and key genes

To explore the possible mechanisms of regulation between miR-155-5p and its target genes, we acquired the sequence of

miR-155-5p and the three key genes from miRbase and NCBI–nucleotide. The binding sites were predicted by RNA hybrid with the criteria that mRNA has perfect nucleotide pairing between the second and eighth positions of the 5′ end of miRNA sequence. Furthermore, the character of the binding sequence was also investigated.

CONCLUSION

This study confirmed a significant upregulation of miR-155-5p expression in cervical cancer by meta-analysis with the data from TCGA and GEO database. The expression of miR-155-5p is significantly correlated with the number of tumour purity and histopathological grades. Furthermore, miR-155-5p promotes the progression of cervical cancer by targeting at least four key genes (SP1, EGFR, UBR4 and PIk3R1) through two crucial pathways (estrogen signaling pathway and choline metabolism). It is possible that miR-155-5p can prevent TTP from binding to the mRNAs in the 3′UTR and therefore regulate their expression. Our findings delineate the role of miR-155-5p and its possible targets in cervical cancer based on *in silico* integrated studies. However, the specific mechanisms of gene regulation need to be further investigated in experimental settings that may help gain insights for future directions for therapeutic opportunities.

Data accessibility

All data generated in this study will be available from the corresponding author on reasonable request. Correspondence and request for materials should be addressed to B.C.D (bcdas@amity.edu; bcdas48@hotmail.com) or D.K. (: dkumar13@amity.edu, dhruvbhu@gmail.com)

Author contributions

M.A. designed the study and performed the acquisition, analysis, and interpretation of data. M.G. assisted with the manuscript writing. D.K. supervised the study, performed a critical revision of the manuscript, checked analysis and interpretation of data. B.C.D critically revised the manuscript for intellectual content.

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Conflicts of interest

The authors declare that they have no conflict of interests.

SUPPLEMENTARY INFORMATION

Supplementary material sheet is provided with Table S1 Characteristics of key genes .

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