RESEARCH ARTICLE

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The Immune-related ceRNA Network in Prognosis of Cervical Cancer

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Abstract

Background: Immunotherapy is gaining attention and it is being included as one of the treatment strategies for cancer patients. However, the molecular mechanisms of immune-related genes and their affinity for cervical cancer progression remain unclear. In this study, we have developed an immune-related competing endogenous RNA [ceRNA] network and assessed the tumour infiltrating immune cells towards the prognosis of cervical cancer. **Methods:** Differential RNA expression pattern between stages I and II-IV of cervical cancer patients from The Cancer Genome Atlas [TCGA] was analyzed. Immune-related ceRNA network based on the immune gene signatures were retrieved and their targets were predicted using miRwalk 3.0. CIBERSORT was employed to identify the immune cell types based on their respective transcripts. The prognostic significance of RNAs in the ceRNA network and immune cell subsets was analyzed. **Results:** Significant differences in 22 long non-coding RNAs [lncRNAs], 15 microRNAs [miRNAs], and 252 messenger RNAs [mRNAs] between stages I and II-IV of cervical cancer were observed. Further, we shortlisted the 49 immune-related mRNAs based on immune gene signature and predicted their target miRNAs and lncRNAs. A potential ceRNA network of 4 lncRNAs, 10 miRNAs, and 11 mRNAs had a strong correlation for prognosis. Out of 11 protein-coding immune mRNAs, IRF4 and AZGP1 had high degrees of interaction. In addition, the evaluation of immune cell subsets showed increased infiltration of M1 macrophages had better survival outcome. **Conclusions:** We have identified an immune-related ceRNA network based on differentially expressed transcripts between stages I and II-IV which may help predict the prognosis of cervical cancer.

Keywords: Cervical cancer- ceRNA network- Prognosis- Immune-related genes

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Introduction

Cervical cancer is the second most common cancer occurring in females, with a high mortality rate in India (Bray et al., 2018). The primary treatment for early-stage cervical cancer includes surgery, radiotherapy, or chemotherapy, and the advanced stage treatment involves concurrent chemoradiation followed by surgery. Stage-associated immune imbalance plays a major role in the progression of cervical cancer, particularly imbalance in Th1/Th2 and Th17/Tregs involved in the development of cervical cancer (Lin et al., 2020). Therefore, it is critical to investigate molecular mechanisms involved in the development and progression of cervical cancer which may help in strategizing newer therapies.

Immunotherapy is being established as one of the treatment modalities for cancer and it is developing (Stanculeanu et al., 2016). Currently, PD-L1 based immunotherapy is widely used to treat various cancer patients. However, fewer than 20% of the patients benefit from this treatment (Sui et al., 2018), which indicates only a subset of the population is prone to the immunotherapeutic intervention. Therefore, finding indicators to evaluate immune infiltration and the examination of their potential mechanisms will aid in the progression of immunotherapy.

The multifaceted role of different RNAs such as mRNA, miRNA, cirRNA, and lncRNA in the progression of cancers has been studied (Sun and Kraus, 2015). Many studies have demonstrated that the interaction of miRNA and lncRNA in deregulating mRNAs [competing endogenous RNAs – ceRNAs] results in the development and progression of cancers (Sun et al., 2020). The possible cross-talk between miRNA-lncRNA-mRNA has been widely studied (Cao et al., 2017; Lopez-Urrutia et al., 2019), and ceRNA network analysis has been established to be an effective method of screening potential prognostic biomarkers in varied cancer types (Zhang et al., 2016). Jiang et al (2020), in their study, constructed a ceRNA regulatory network with 48 lncRNAs, 14 miRNAs, and 4mRNAs and further, they explored that ceRNAs play a crucial role in gene regulation and these genes could

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be used as reliable prognostic predictors of bladder cancer (Jiang et al., 2020). In addition, the aberrant regulation of the ceRNA network also plays a key role in tumorigenesis and the progression of multiple cancers, such as gastric cancer (Qi et al., 2020), breast cancer (Gao et al., 2019) and ovarian cancer (Li et al., 2020). In addition, a recent study comparing normal (healthy controls) vs. cervical cancer showed the immune-related ceRNA network involved in the prognosis of cervical cancer (Zheng et al., 2020). Although several researchers have implicated the role of these ceRNAs in multiple cancers, specific studies on ceRNAs involved in the regulation of mRNAs in infiltrating immune cells in cervical cancer are limited. So, RNA sequencing data at different cervical cancer stages were downloaded from The Cancer genome atlas [TCGA] database to comprehensively understand the ceRNA network during disease progression. More specifically in this study, we tried to identify the role of lncRNAs and miRNAs interacting with the mRNAs involved in immune-related functionality of cervical cancer between stage I vs. stage II-IV.

Materials and Methods

Identification of differentially expressed RNAs

We retrieved Htseq raw counts and clinical information of 300 Cervical Squamous Cell Carcinoma [CESC] from TCGA using TCGAbiolinks Rv4.0/Bioconductor package (Mounir et al., 2019). The obtained data were categorized into two groups stage I [n=171] and stage II-IV [n=126], normal [N=3] were excluded from the analysis. The GDCRNATools were utilized to identify the differentially expressed [DE] mRNAs, lncRNAs, and miRNAs through edgeR package between stages I and II-IV (Li et al., 2018). We filtered the genes with fold change >1 and <-1 and p-value <0.05, to identify robust gene candidates in all the categories. P values were adjusted for multiple comparisons using the false discovery rate [FDR] procedure of Benjamini and Hochberg (Benjamini, 2010).

Target prediction for miRNAs

miRNA-targeted lncRNAs was predicted using miRwalk v3.0 (Sticht et al., 2018) contains other tools such as miRanda (Betel et al., 2010), RNAhybrid (Kruger and Rehmsmeier, 2006), and TargetScan (Agarwal et al., 2015), the targets were retained if they were identified by 3 out of 4 algorithms. Secondly, miRNA-targeted mRNAs were predicted based on the potential targeted regions [e.g. the 3' and 5' untranslated regions [UTRs]] using miRwalk 3.0, and matched targets were retained as candidates if they were identified by 6 out of the 12 algorithms [3'UTR] and 5 out of 12 algorithms [5'UTR]. The list of 12 algorithms includes DIANA-microT, DIANA- micorT-CDS, miRanda, mirBridge, miRDB, miRmap, miRNA Map, PicTAR2, PITA,RNA22, RNAhybrid, and Targetscan. Based on this analysis, we predicted a list of potential miRNA which are regulating both lncRNAs and mRNAs.

Immune-related ceRNA network

Immport database[https://www.immport.org/shared/genelists] (Bhattacharya et al., 2018) contains genes specific to cytokines, immune receptors along with gene signatures implicating the specific immune cell types from CIBERSORT (Newman et al., 2015) and xCell (Aran et al., 2017) were downloaded and pooled to study the mRNAs involved in ceRNA network. The Cutoff point for transcripts was obtained using R package survminer v 0.4.9 (Alboukadel Kassambara). The predicted DE RNAs were used as input to construct a ceRNA network using Cytoscape v.3.7.2.

Cell enrichment analysis using CIBERSORT

Normalized gene expression data of CESC were used to infer the relative proportions of 22 types of infiltrating immune cells using the CIBERSORT algorithm as previously reported (Newman et al., 2015). The data was uploaded to the CIBERSORT web portal [http://cibersort.stanford.edu/], and run using the LM22 signature matrix at 1000 permutations. Cut off for the immune cell subsets were set using Xtile software v 3.6.1 (Camp et al., 2004). The overview of the work carried out was given in the form of a flow chart in supplementary Figure 4.

Statistical analysis

Survival outcomes were calculated using clinical data downloaded from TCGA-CESC. The transcripts were stratified into low and high based on expression levels using the survminer cut-point analysis using R. This is an outcome-oriented method providing a value of a cutpoint that corresponds to the most significant relationship with the survival. Survival curves for DEmiRNAs, DElncRNAs, DEmRNAs, and different immune cell types were constructed using the Kaplan-Meier method. For univariate and multivariate analysis of prognostic variables, Cox proportional hazard [PH] model was applied and the variable with p-values< 0.05 was considered significant. TIMER software was used to analyze the correlation of gene expression with immune cell types (Li et al., 2017). All statistical analyses were performed using SPSS V.17; SPSS Inc., Chicago, IL, USA, Graphpad prism v 7 [GraphPad Software, Inc. USA], and R package survival version 3.2-11 (Terry M Therneau, 2022).

Results

Differentially expressed lncRNAs, miRNAs, and mRNAs in CESC

To better understand prognosis-associated differentially expressed lncRNAs, miRNAs, and mRNAs, we did a comparison between the stage I and II-IV tumors (supplementary Figure1 a-c). We identified 252 differently expressed mRNAs [unregulated -121, downregulated-131], 22 lncRNAs [upregulated-7, downregulated-15] and 15 miRNAs [upregulated-10, down regulated-5] (supplementary Figure 1d, supplementary Table 1). Further differentially expressed RNAs were used for constructing a ceRNA network and to identify genes that are involved in immune regulation. ceRNA network comprises lncRNA which sponges miRNA, thereby it competes and shares mRNA forming an interactive RNA network. Identification of this network may aid in

Table 1. Univariate and Multivariate Cox Regression Analysis of RNAs Involved in ceRNA Network

			Univariate analysis			Multivariate analysis		
Variables	Factors	Cases	HR	95% CI	p value	HR	95% CI	p value
ENTDP1-AS1	High	207	1.00[a]		0.017*	1.00[a]		0.109
	Low	58	0.386	0.177-0.845		0.516	0.230-1.159	
MUC2	High	31	1.00[a]		0.029*	1.00[a]		0.034*
	Low	234	0.521	0.289-0.937		0.523	0.288-0.951	
DLX6-AS1	High	187	1.00[a]		0.028*	1.00[a]		0.025*
	Low	78	0.515	0 .285- 0.930		0.505	0.278-0.917	
CTBP1-AS	High	100	1.00[a]		0.001*	1.00[a]		0.019*
	Low	165	0.463	0.288-0.745		0.556	0.339-0.909	
miR-940	High	132	1.00[a]		0.056*	1.00[a]		0.899
	Low	133	1.596	0.987-2.576		0.954	0.463-1.964	
miR-3130	High	39	1.00[a]		0.016*	1.00[a]		0.444
	Low	226	2.811	1.210-6.535		1.453	0.558-3.781	
miR-326	High	53	1.00[a]		0.037*	1.00[a]		0.877
	Low	212	2.055	1.045-4.039		1.067	0.469-2.428	
miR-3677	High	155	1.00[a]		0.007*	1.00[a]		0.257
	Low	110	1.916	1.191-3.083		1.517	0.738-3.118	
miR-149	High	208	1.00[a]		0.031*	1.00[a]		0.105
	Low	57	1.784	1.053-3.025		1.632	0.903-2.949	
miR-134	High	29	1.00[a]		0.023*	1.00[a]		0.075
	Low	226	5.133	1.257-20.967		3.964	0.870-18.069	
miR-654	High	77	1.00[a]		0.045*	1.00[a]		0.48
	Low	188	1.824	1.013-3.285		0.783	0.397-1.544	
miR-194	High	169	1.00[a]		0.01*	1.00[a]		0.482
	Low	96	1.862	1.158-2.994		1.281	0.642-2.558	
miR-9	High	232	1.00[a]		1E-04*	1.00[a]		0.006*
	Low	33	2.768	1.570-4.880		2.348	1.272-4.336	
miR-192	High	143	1.00[a]		0.002*	1.00[a]		0.114
	Low	122	2.168	1.331-3.530		1.774	0.871-3.612	
HGF	High	211	1.00[a]		0.023*	1.00[a]		0.742
	Low	54	1.834	1.087-3.095		1.119	0.573-2.186	
AZGP1	High	167	1.00[a]		0.001*	1.00[a]		0.001*
	Low	98	0.384	0.216-0.681		0.37	0.206-0.682	
PDK4	High	98	1.00[a]		0.042*	1.00[a]		0.206
	Low	167	1.735	1.021-2.947		1.541	0.788-3.013	
ALDH1A2	High	135	1.00a		0.026*	1.00[a]		0.375
	Low	130	1.732	1.069-2.807		1.318	0.716-2.427	
TMEM255A	High	223	1.00[a]		0.036*	1.00[a]		0.039*
	Low	42	0.408	0.176-0.945		0.39	0.161-0.953	
CDHR1	High	75	1.00[a]		1E-05*	1.00[a]		0.081
	Low	190	0.409	0.254-0.658		0.612	0.353-1.063	
FAM3D	High	137	1.00[a]		0.011*	1.00[a]		0.066
	Low	128	0.524	0.319-0.861		0.598	0.345-1.034	
CDA	High	70	1.00[a]		0.009*	1.00[a]		0.69
	Low	195	0.52	0.319-0.848		0.892	0.510-1.561	
ACKR2	High	41	1.00[a]		0.016*	1.00[a]		0.291
	Low	224	3.09	1.237-4.714		1.701	0.634-4.563	
IRF4	High	212	1.00[a]		0.028*	1.00[a]		0.148
	Low	53	1.803	1.065-3.053		1.625	0.841-3.139	
CSF3	High	119	1.00[a]		0.016*	1.00[a]		0.074
	Low	66	0.437	0.223-0.856		0.523	0.257-1.064	

a, Reference category; HR, Hazard ratio; CI, Confidence interval; *, P - < 0.05 The highlighted transcripts indicates significance in both univariate and multivariate analysis

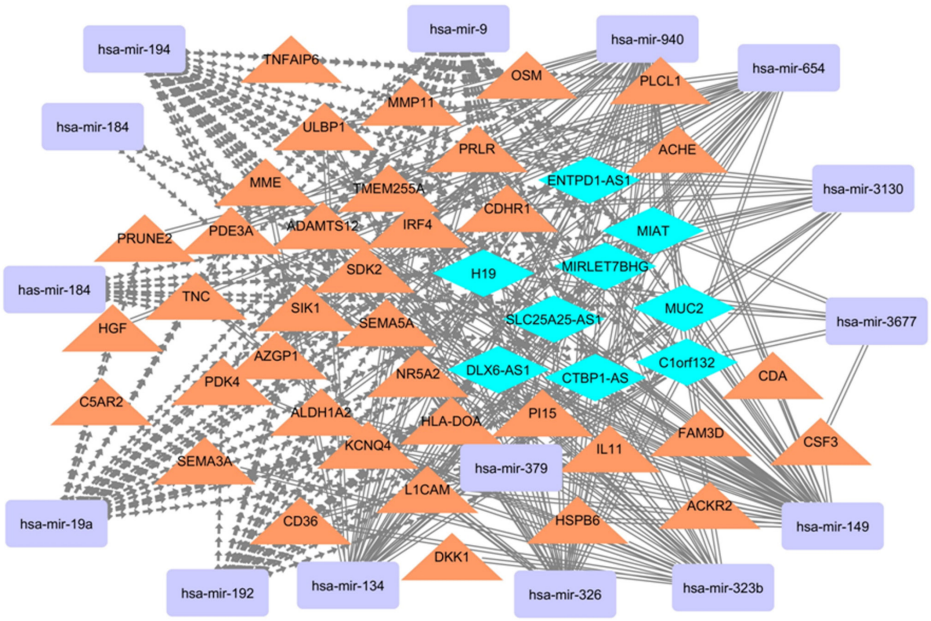

Figure 1. Immune-related Competing Endogenous RNA [ceRNA] Network Comprising of 9 - lncRNAs [Cyan] -,14 – miRNAs [Purple], 37 – mRNAs [Orange]. The solid line indicates the interaction of upregulated miRNA, whereas the dotted line indicates the interaction of downregulated miRNA with mRNA and lncRNA.

predicting the prognosis of cervical cancer patients.

Prediction of miRNA targeted lncRNAs and mRNAs involved in immune regulation

From 252 differentially regulated mRNA, 49 immune-related mRNAs were retrieved using Immport database, CIBERSORT, and XCell. Deconvolution tools CIBERSORT and XCell aid in identifying the immune cell types from bulk RNA seq datasets. We also utilized the gene signature of those tools along with immport gene sets to retrieve maximum immune transcripts out of DE RNAs. We predicted the interaction of these 49 mRNAs with the 22 DElncRNA and 15 DEmiRNA using miRwalk3.0. The target prediction showed 14 miRNAs had interaction with 9 lncRNA [9/22DElncRNAs] and 37 mRNAs [37 / 49 immune-related mRNAs] (Figure 1).

Survival and prognostic analysis of DE RNAs associated with immune ceRNAnetwork

K-M survival analyses with log-rank test for each gene were performed. The analysis revealed high expression of 4lncRNAs and 10mRNAs were negatively correlated with overall survival [$p<0.05$]. Additionally, the expression levels of 5mRNAs and 10miRNAs were positively correlated with the overall survival of patients. Univariate Cox PH analysis for each DERNA [9lncRNAs, 14miRNAs, 37mRNAs] was performed. 4 lncRNAs [ENTDP1-AS1, MUC2, DLX6-AS1, CTBP1-AS1] and 6mRNAs [AZGP1, TMEM255A, CDHR1, FAM3D, CDA, CSF3] were shown to be related to poor survival. While, 10miRNAs [miR940, miR3130, miR326, miR3677, miR149, miR134, miR654, miR194, miR9, and

Table 2. Univariate and Multivariate Cox Regression Analysis of Tumour Infiltrating Immune Cell Subsets Such as aNK, M0, M1 and Neuttrophils

			Univariate analysis			Multivariate analysis		
Variables	Factors	Cases	HR	95% CI	p value	HR	95% CI	p value
Activated Natural Killer	High	50	1.00[a]		0.01*	1.00[a]		0.063
	Low	107	0.436	0.226-0.841		0.524	0.266-1.035	
M0 macrophages	High	50	1.00[a]		0.03*	1.00[a]		0.078
	Low	107	0.48	0.250-0.922		0.547	0.280-1.070	
M1 macrophages	High	114	1.00[a]		0.00*	1.00[a]		0.005*
	Low	43	3.718	1.925-7.179		2.801	1.368-5.737	
Neutrophils	High	11	1.00[a]		0.01*	1.00[a]		0.132
	Low	146	0.228	0.076-0.685		0.412	0.130-1.304	

a, Reference category; HR, Hazard ratio; CI, Confidence interval; *, P - < 0.05 The highlighted cell type indicates significance in both univariate and multivariate analysis

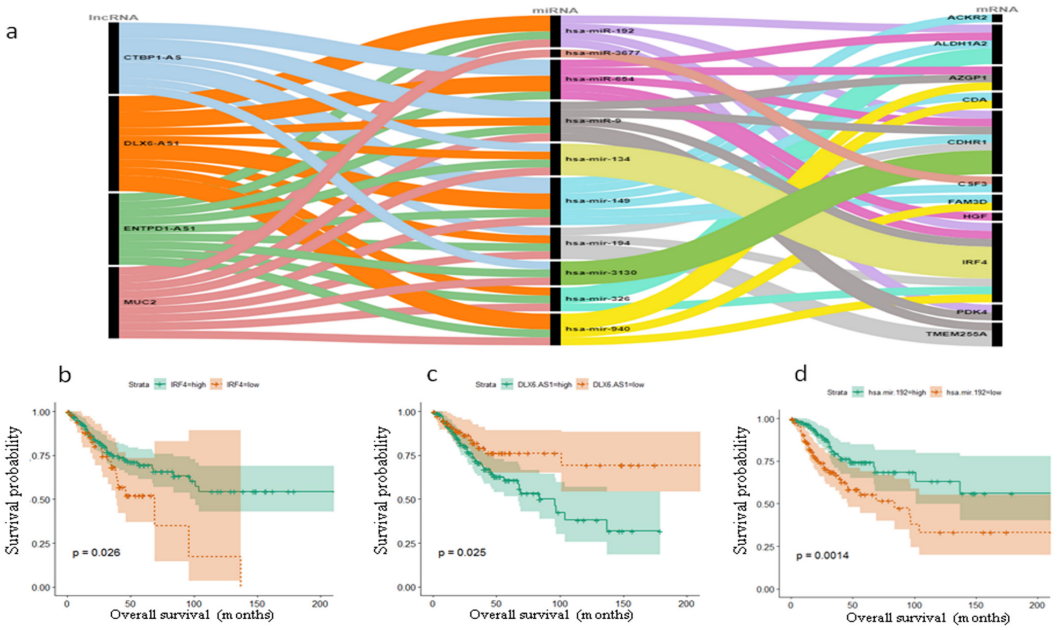

Figure 2. Prognostically Significant ceRNA Network. (a) Alluvial plot represents identified ceRNA network in cervical cancer. Each rectangle represents identified RNAs and their interaction with each other. The size of each rectangle indicates the degree of interaction. (b-d) Kaplan-Meyers plot for top mRNA[IRF4], lncRNA[DLX6-AS1] and miRNA[has-miR-192]. The p-value of <0.05 was considered significant.

miR192] along with 5mRNAs [HGF, PDK4, ALDH1A2, ACKR2, and IRF4] were found to be associated with good prognosis. Significant variables from the univariate analysis were assessed using the multivariate Cox PH model, out of which 3 lncRNAs, 1 miRNA, and 2 mRNAs were found to be independent predictors of prognosis. The Cox proportional hazard analysis for the lncRNA-miRNA-mRNA network is presented in Table 1. The Alluvial plot was constructed using significant DE RNAs [4LncRNA, 10miRNA,11mRNA] derived from the univariate Cox PH model (Figure 2a). The K-M survival curves of top-ranked lncRNA [DLX6-AS1], miRNA [miR-192] and mRNA[IRF4] based on the significance are shown in (Figure 2b – d).

Identification of key immune mRNAs involved in cervical cancer progression

As a next step, we sought to identify the hub genes which might involve in regulating more genes. Hence, the 11 mRNAs with prognostic significance were taken into the OmicsNet database (Zhou and Xia, 2019) to identify their protein interacting partners. IRF4 and AZGP1 were found to have a high degree of interaction [≥ 10]. To further explore the interacting partners of IRF4, STRING database v 11.0 (Szklarczyk et al., 2017) was utilized to check the interactive partners ofIRF4 [21 nodes, 92 edges] and AZGP1[21 nodes, 41 edges] (Figure 3a and b). Additionally, correlation analysis using TIMER software [which provides information only for the mRNA] revealed CD8+ T-cells, CD4+ T-cells, B-cells, dendritic cells, and neutrophils had a positive correlation with IRF4 gene whereas, AZGP1 had a positive correlation with B-cells (Figure 3 c and d and supplementary Figure 2).

Immune cell enrichment analysis

Univariate Cox PH analysis using 22 immune cells subsets obtained from CIBERSORT showed infiltration of Macrophages [M1] was associated with better prognosis whereas, activated NK cells, Macrophages M0, and neutrophils were found to negatively correlate with survival. In addition, the multivariate analysis also identified M1 macrophage as an independent predictor of good prognosis. Table 2 shows univariate and multivariate Cox PH analysis of tumour infiltrating immune cell subsets.

Discussion

Cervical cancer is considered as second most common gynecological malignancy among women in India. The incidence rate of cervical cancer has decreased worldwide, but the mortality is still high in developing countries including India (Bray et al., 2018). Although there is an improvement in the treatment of patients presenting at a locally advanced stage, approximately 50% experience recurrence within the first two years (Bandyopadhyay et al., 2018). The development of a new treatment method is therefore necessary. Immunotherapy is being considered as an additional treatment strategy in several cancers including cervical cancer. In this study, we retrieved RNAseq data from TCGA– CESC dataset

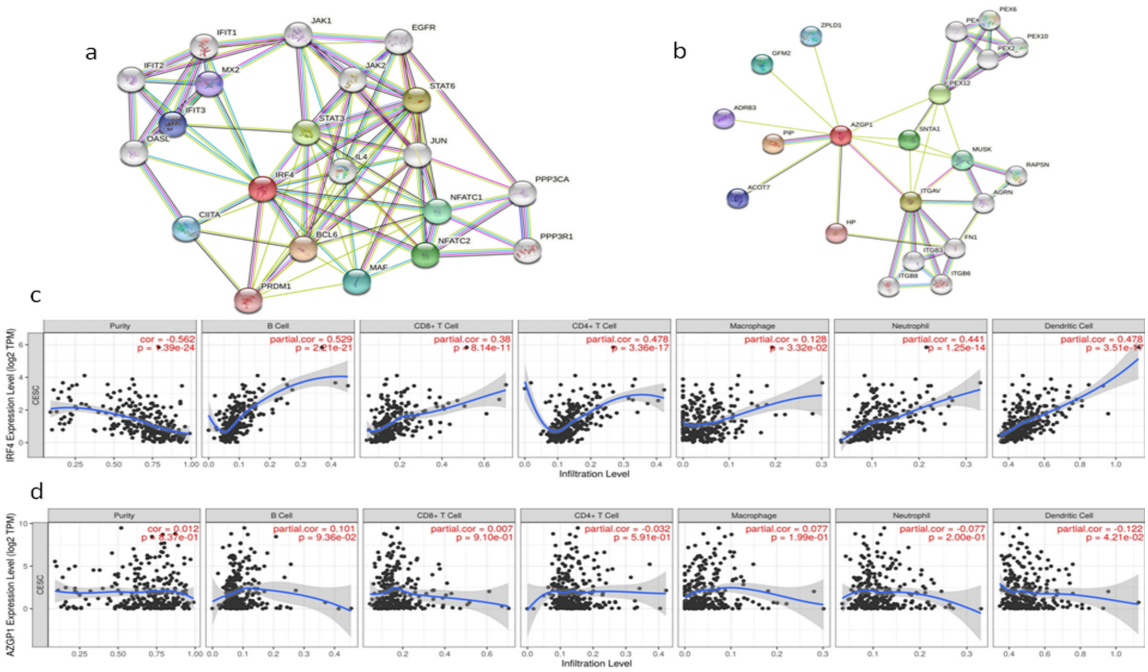

Figure 3. Immune Genes are Involved in Cervical Cancer Progression. Protein-protein interaction network of (a) IRF4 and (b) AZGP1.Correlation plot for (c) IRF4 and (d) AZGP1 in relation with immune cell subtypes. The p-value of <0.05 was considered significant.

and established immune-related lncRNA-miRNA-mRNA ceRNAnetwork through computational prediction.

The ceRNA network showed miR-149 which was upregulated in stage I interacts with 9 lncRNAs and 20 mRNAs. The role of miR-149 was much pronounced in inhibiting the Epithelial-mesenchymal transition (EMT) of tumour which plays a vital role in the invasion and metastasis properties of cancer cells. Mounting evidence showed the up-regulation of its expression correlated with inhibition of metastasis by down-regulating Forkhead box M1 (Okato et al., 2017) and MMP-2 (Pan et al., 2012) expression via blocking the AKT1 signaling. In addition, miR-194 which was downregulated in stage I interacts with 8 lncRNA and 18 mRNAs. However, the increased expression of miR-194 correlated with better survival in endometrial cancer (Zhai et al., 2013). Inline our study showed the increased expression of DE-miR which is involved in the ce-RNA network revealed a better prognosis for the patients.

Many studies have shown that the increased expression of DLX6-AS1 corelates to poor prognosis in glioma and colorectal cancer (Li et al., 2019; Zhang et al., 2019). Meng Zhou et al. showed the role of immune-related six lncRNA signatures including the ENTPD1-AS1 could stratify patients with low and high-risk groups in glioblastoma (Zhou et al., 2018). In addition, a recent study showed the overexpression of lncRNA CTBP1-AS increased the breast cancer cell proliferation and inhibition of cell apoptosis by sponging miR-940 (Cui and Geng, 2019).

Our ceRNA network prediction revealed 4 lncRNA [ENTPD1-AS1, MUC2, DLX6-AS1, and CTBP1-AS] and 7miRNAs [miR134, miR326, miR940, miR194, miR192, miR9 and miR654] involved in the regulation of IRF4. While, 4lncRNA and 3miRNAs [miR9, miR654, and miR940] were found to be regulating AZGP1. Identified lncRNA-miRNA-mRNA network may play a potential role in the survival and progression of cervical cancer. To this end, we have done a survival analysis and Cox PH estimate for the identified network. LncRNAs with high expression had a poor prognosis whereas increased expression of miRNAs had a better survival outcome.

Competing endogenous RNA [ceRNA] including circular RNAs and lncRNAs having shared miRNA response elements [MREs] influences by competing for the miRNAs thereby modulating the expression of mRNAs. The regulatory mechanisms between lncRNAs and miRNAs are extremely complex (Mitra et al., 2018). Among them, lncRNA as a part of ceRNA network sponges miRNA to compete and share mRNA, thus forming a complex lncRNA-miRNA-mRNA [ceRNA] network.

Arianna Mangiavacchi et al., (2016) showed the importance of ceRNA linc-223 which sponges miR-125-5p and causes upregulation of IRF4 wherein the knockdown of linc-223 increases the repressing activity of miR-125-5p leading to downregulation of IRF4. In addition, a study showed that IRF4 acts as a key regulator in lymphoid, myeloid, and dendritic cells and mature B-cells differentiation. HPV- E7 mediated hypermethylation of IRF4, FOXF1 and glucocorticoid receptor TF motifs near immunoregulatory and developmental genes may cause the progression of cervical cancer (Cicchini et al., 2017). Our study showed that the relatively lower expression of IRF4 indicated a poor prognosis of cervical cancer which is in agreement

with the previous study (supplementary Figure 3). AZGP1 plays an antitumor role by participating in immune response, due to its high structural homology to the major histocompatibility complex class I family (Tang et al., 2017). Downregulation of AZGP1 was also associated with poor prognosis in many tumour types such as breast cancer, prostate cancer, esophageal squamous cell carcinoma, and gastric cancer. Increasing evidence suggests the association of lncRNA with gene regulation. A recent report showed lncRNAs XLOC_010739 and G015949 were associated with downregulation of genes including AZGP1 resulting in poorer prognosis in hepatocellular carcinoma [HCC] (Lim et al., 2020). In contrast, our study showed relatively lower expression of AZGP1 associated with a better prognosis of cervical cancer, which needs further validation.

The importance of tumour and immune cells' cross-talk and their implication in tumour prognosis has been reported in several studies (Wang et al., 2019). Tumour-associated macrophages constitute a substantial fraction in all cancer. Non-polarised M0 macrophages are highly pleiotropic and capable of changing their phenotype to M1 or M2 depending on the tumour microenvironmental signals. A recent study showed the differentiation of M0 to M2-like phenotype when stimulated with apoptotic SKVO3 cell line promotes tumour proliferation and migration (Zhang et al., 2020). Whereas, the presence of M1 polarised macrophage was involved in efficient tumour lysis and acts as potential antigen-presenting cells. They also support the Th1 response by recruiting the cytotoxic T cells; thereby it promotes a potent anti-tumour response (Maccio et al., 2020).

Meta-analysis data of 3946 patients with varied solid tumours from 20 studies revealed an increased density of intratumoral neutrophils, independently, associated with a poor prognosis of cancer (Shen et al., 2014). NK cells act as the first line of anti-tumour defence by providing tumour lysis and elimination of tumour metastasis (Lopez-Soto et al., 2017). However, the presence of TGF-β alters the degranulation capability of NK cells causing upregulation of vascular endothelial growth factor [VEGF], placental growth factor [PGF] production resulting in the pro-angiogenic phenotype of NK cells (Bruno et al., 2013). Inline our study also showed that an increase in M0, neutrophils, and NK cells were associated with poor prognosis whereas the increase in M1 showed favorable survival.

In conclusion, we developed a ceRNA network based on transcripts obtained from differential expression between stages I and II-IV cervical cancer. Immune-related genes were then retrieved and independently analyzed for K-M survival and the Cox PH model. The predicted ceRNA network provides comprehensive data on cancer prognosis. Further, this study shed light on the importance of tumour-associated immune cell subtypes and their role in predicting the prognosis of cervical cancer and opens up a newer avenue for immunotherapeutic strategies. The findings of this study are limited as the identified markers are from bulk RNAseq datasets, which ideally necessitates it to be further validated in immune specific single cell RNAseq datasets and also in patients' samples with longer follow-up data.

Author Contribution Statement

H.D carried out all the analysis, prepared figures, and drafted the manuscript. M.B was involved in data analysis, preparation of figures and critical review of the manuscript. S.K designed the study, involved in data analysis, preparation, and review of manuscript. All authors approved the final version of the manuscript.

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Declaration of competing interest

The authors declare no competing interests.

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