Original Article

A Cancer-Array Approach Elucidates the Immune Escape Mechanism and Defects in the DNA Repair System in Esophageal Squamous Cell Carcinoma

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is the second-most frequently diagnosed cancer in Northeast Iran, often diagnosed in advanced stages. No standard early diagnostic guideline has been proposed to date and current therapeutic modalities are not effective. Detection of tumor-specific biomarkers, which is the goal of this study, could prove useful in the diagnosis of ESCC.

Methods: To better understand the gene expression profile of ESCC, we analyzed tumor samples and corresponding adjacent normal tissues from ESCC patients by Chemiluminescent Human Cancer GEArrays. Candidate genes were verified by real-time PCR.

Results: Out of 440 cancer-related genes included in the array, 71 were overexpressed compared to normal tissue, with significant differences in 11 genes. There were 108 genes underexpressed, with significant differences in 5 genes. Until now, the AP2M1, FTL, UBE2L6, HLA-C, and HSPA8 overexpressed genes and XRCC5, TP53I3 and RAP1A underexpressed genes were not reported in ESCC. We chose the MMP2, HLA-G, and XRCC5 markers from 58 Iranian ESCC patients to verify the expression validity by real-time PCR. The microarray results were confirmed with two-tailed significance levels of $P = 0.003$ (MMP2), $P = 0.000$ (HLA-G) and $P = 0.002$(XRCC5). Analysis performed for the candidate genes using GNCpro online software highlighted two pathways, an immuno-modulatory response and DNA replication and repair. We successfully performed and validated Chemiluminescent GEArray gene expression profiling in ESCC. Several biomarkers that might be related to tumorigenesis in ESCC were identified.

Conclusion: Immuno-modulatory and DNA repair pathways could be used as targets to locate specific diagnostic, prognostic, and therapeutic biomarkers for ESCC.

Keywords: Biomarker, DNA repair, ESCC, gene expression, immune escape, profiling

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Introduction

Esophageal cancer is the seventh most common cause of cancer deaths worldwide, and the fifth leading cause of cancer-related death in Iran.[1,2] Esophageal squamous cell carcinoma (ESCC) comprises 95% of all cases.[1] There is a wide variation in the epidemiology of this cancer, ranging from 3 per 10^5 person-years amongst American Caucasians to more than 100 per 10^5 person-years in some areas of China.[3–6] The high incidence areas of Iran include Golestan and Khorasan Provinces in Northeastern Iran with age standardized rates (ASR) of 43.4 per 100,000 for men and 36.3 per 100,000 for women.[7]

These regions of Iran are located in a high-risk area designated as the "Central Asian Esophageal Cancer Belt".[8] The geographic distribution of ESCC incidence varies greatly based on environmental and genetic factors.[3,9,10] Investigators have shown a 20-fold difference in the incidences of ESCC between high-risk China and low-risk western Africa.[4]

Esophageal cancer patients have low survival rates; only 16% of cases in the United States[11] and 10% in Europe[12] survive for five years. The five-year survival rate for ESCC patients has been reported to range from less than 10%[13] to 25%–30%.[14,15] Treatment success depends on early diagnosis. Because ESCC is asymptomatic until late stages of the disease, successful surgical treatment is low. To date, various environmental risk factors have been proposed for ESCC; however, specific underlying genetic alterations have not been well-defined for patients in Northeastern Iran.[8,16] Thus, identification of new molecular diagnostic markers for ESCC would likely be beneficial because early detection and treatment lead to increased survival rates.

Several studies were performed to find early diagnostic biomarkers for ESCC.[17–20] Our group showed that noninvasive methods such as analysis of aberrant P16 methylation in serum and blood of patients with ESCC might be a useful diagnostic marker for high-risk populations.[21] In addition, multiple gene hypermethylation analysis showed that overexpression of DNMT3b could be

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involved in ESCC pathogenesis; thus, cluster analysis of multiple methylated genes in serum DNA might be a novel method for the diagnosis of ESCC.[22]

Gene expression profiling, widely utilized in expression genomics, is a promising way to locate new biomarkers for cancer detection and treatment. Microarray is the fundamental basis of expression genomics. As hundreds or thousands of DNA probes are spotted on a solid surface, genetic analysis can be accomplished in parallel. Fluorescent or chemiluminescent-labeled cDNA or cRNA from target RNA sources can be hybridized against chips.[23] Microarray studies have many advantages, including introduction of newly-expressed genes with unknown roles, determining relationships among multiple genes, defining highly specific patterns of gene expression that assist in characterizing particular disease stages, and prognosis or treatment.[24] Gene expression profiling of ESCC has been performed by different investigators in China,[18,25,26] Japan,[27] India,[19] and the Netherlands[28]; however, these studies have not identified any definite markers for esophageal cancer.

To date, no microarray study has been performed in Iran to find potential biomarkers for ESCC. The goal of the present study was to identify potential altered biomarkers and pathways in Iranian ESCC using chemiluminescent microarray membranes.

Materials and Methods

Sample collection

This study was approved by the Research Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran. We enrolled patients with ESCC who had no history of prior chemotherapy or radiotherapy following informed consent. Immediately after surgery, three 1 mm × 0.8 cm^2 segments of resected tumor were prepared by a pathologist. One segment was transferred to RNAlater (Qiagen, Germany) for molecular analysis; the remaining two segments were placed in formalin for Hematoxylin and Eosin (H&E) staining in order to verify the presence of ESCC and confirm a high tumor cell percentage in the sample (>70%). From the furthest part of the esophagus, according to tumor location, we excised several 1 mm^3 mucosal specimens and transferred them to RNAlater as the normal margin.

Expression array

RNA from tumor and normal margin tissues of each patient was extracted using an RNA extraction kit (Qiagen, Germany). The quality of the RNA samples was assessed both spectrophotometrically and by a ribosomal RNA integrity assay. In all samples, the A260:A280 ratios were greater than 2.0 and the A260:A230 ratios were greater than 1.7. For microarray experiments, cDNA was synthesized using 3 μg of starting RNA with a TrueLabeling RNA kit (SABiosciences, USA) according to the manufacturer's instructions. To produce biotinylated cRNA, cDNA was incubated with cRNA synthesis master mix for 18 h at 37°C (Truelabeling RNA kit, SABiosciences, USA). To remove protein and excess unincorporated nucleotides, cRNA was further purified by a cRNA clean up kit (SABiosciences, USA). cRNA quantification was performed by spectrophotometry (Amersham Pharmacia, Biotech).

The OligoGEArray® Human Cancer biomarker microarray kit was used to analyze the expression profile (SABiosciences, USA). The chemiluminescent cDNA microarray consisted of 480 spots that comprised 440 cancer-related genes, 40 housekeeping genes for normalization, and control spots for quality control. The cancer-related genes belonged to several different pathways frequently altered during cancer progression. Most commercial arrays contain these genes; hence, by selecting this array it was possible to compare the results of this study with other studies. For microarray experiments we analyzed the tumors and matched adjacent normal tissues from five patients with ESCC. The purified cRNA was hybridized to the Human Cancer GEArrays (HybPlate Basic Protocol, SABiosciences, USA). Hybridizations were performed at 60°C in multi-chamber hybplates for 20 hr. After washing, image acquisition was performed with Syngene gel documentation (Syngene, UK). We analyzed the resultant raw images by GEArray Expression Analysis Suite (Online Image Data Acquisition and Analysis Software). The software identified both over- and underexpressed genes that had P-values ≤ 0.05.

Verification assay

Real-time PCR was used to verify selected significantly-altered markers from the microarray analysis. MMP2 and HLA-G from overexpressed genes and XRCC5 from underexpressed genes were evaluated. GAPDH was used as an internal control. IL-10 was also chosen to test our hypothesis of immune escape in ESCC according to previous data.[29,30] We used the following primers, as described in Table 1: XRCC5,[31] MMP2,[32] HLA-G,[33] IL10,[30] and GAPDH[30] (MWG, Germany). Experiments were performed in duplicate for 58 ESCC patients. The SYBR Green comparative method was selected for gene expression analysis in tumor and matched normal tissues. Gene expression was quantified using the Stratagene Mx-3000P real-time thermocycler (Stratagene, La Jolla, CA, USA). We prepared the reaction mix by adding 10 μL of SYBR Green (Fermentas, Lithuania), 0.04 μL of ROX (Invitrogen, Germany), 0.25 μL of each primer (10 pmol), 2 μL of cDNA, and 7.46 μL of nuclease-free water (Fermentas, Lithuania) for a total volume of 20 μL. The thermal cycle conditions were as follows: 50°C for 3 min, then 95°C for 10 min of initial denaturation, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 20 s.

Statistical analysis

We have used the Significance Analysis of Microarrays (SAM) software to re-evaluate the microarray data, which takes into account the repeated measurement nature of the study.[34] Logarithmic transform (fold change) measured the change in the expression level of a particular gene. Using SAM, a delta of 0.71 and a fold change larger than 1.5 were set to discover the significant over- and under-expressed genes. These assumptions gave a median false discovery rate (FDR) of 12.28%. FDR approximates the overall proportion of genes identified by chance (false positives). The q-value for each significant gene was also calculated. The q-value determines how significant a gene is, as with the popular P-value, however it is adjusted to the analysis of a large number of genes and takes into account the FDR. In fact, it is the minimum FDR at which the gene is considered to be significant. All statistical analyses were performed using SPSS 11.5 software for real-time results. In all tests, a P value less than 0.05 was considered significant. The Kolmogorov-Smirnov and Levene's tests were performed to verify normal distribution and the equality of variances, respectively. One sample t-test was used to distinguish the over- and underexpressed genes. The associations be-

Table 1. Primer characteristics for XRCC5, MMP2, HLA-G, IL10 and GAPDH.

Gene	Primer sequence (5' to 3')	Amplicon (bp)	Annealing temp (°C)
XRCC5	F: GCTTTTCCTCATATCAAGCATAACT	247	60
	R: AATCTCTGAAATCGAGGATTTGG		
MMP2	F: CCATGATGGAGAGGCAGACA	85	61
	R: GGAGTCCGTCCTTACCGTCAA		
HLA-G	F: CTGGTTGTCCTTGCAGCTGTAG	79	60
	R: CCTTTTCAATCTGAGCTCTTCTTTCT		
IL10	F: AACCAAGACCCAGACATCAAGG	136	60
	R: CATTCTTCACCTGCTCCACG		
GAPDH	F: GGAAGGTGAAGGTCGGAGTCA	101	60
	R: GTCATTGATGGCAACAATATCCACT		

Table 2. Patients' clinicopathologic characteristics.

Variable	Value	*n* (%)
Sex		
	Male	32 (55.2)
	Female	26 (44.8)
Tumor size		
	Mean ± SD	4.01 ± 1.90
Tumor location		
	Distal	23 (39.7)
	Middle	34 (58.6)
	Proximal	1 (1.7)
pGrade		
	1 (W.D)	12 (20.7)
	2 (M.D)	35 (60.3)
	3 (P.D)	11 (19)
pStage (TNM)		
	I	1 (1.7)
	IIa	31 (53.4)
	IIb	5 (8.6)
	III	21 (36.2)
	IV	0 (0)
pT classification (Depth of tumor invasion)		
	T1	3 (5.2)
	T2	8 (13.8)
	T3	47 (81)
	T4	0 (0)
Lymph node metastasis		
	Positive	24 (41.4)
	Negative	32 (55.2)
	Unknown	2 (3.4)
Survival status		
	Dead	25 (43.1)
	Alive	13 (22.4)
	Unknown	20 (34.5)
Survival time (months)		
	0–12	15/38 (39.5)
	12–24	14/38 (36.8)
	24–36	4/38 (10.5)
	≥ 36	5/38 (13.2)
	Unknown	20

tween different clinicopathological variables and gene expression were examined using the independent sample *t*-test, Pearson and Spearman correlation coefficients, one-way analysis of variances (ANOVA) and chi square, wherever appropriate. We calculated overall patient survival from the date of diagnosis to the date of last follow-up or date of patient death. The Kaplan-Meier method was used to estimate the survival function. Differences in survival times between patient subgroups were investigated using the log-rank test.

Results

This study recruited 32 males and 26 females. Of these, 5 subjects were enlisted for the cDNA microarray study and 58 for the real-time PCR validation experiments. The mean size of the resected tumors was 4.01 ± 1.90 cm. All tumors were located in the distal 23/58 (39.7%) or middle 34/58 (58.6%) esophageal regions with the exception of one proximal tumor 1/58 (1.7%). Most were grade G2, moderately differentiated 35/58 (60.3%) tumors; the majority of cancers were stage II 36/58 (62%). Stage T3 comprised 81% of the tumors, in which the tumor has broken through the adventitia covering the outside of the esophagus. Lymph node metastasis was detected in 24/58 (41.4%) of the samples. Survival data was collected for 38 subjects, however, only 5/38 (13.2%) of this population survived 36 months or more. The clinicopathological characteristics of the subjects are summarized in Table 2.

All tumor tissues chosen for this study were from freshly resected surgical samples and confirmed to have more than 70% tumor cells. RNA extracted from fresh tumor tissues had the required characteristics for the microarray studies. Only three of the five samples yielded expected cRNA concentrations; however, the hybridization experiments were performed for all samples. Based on

Table 3. The summary of significant overexpressed and underexpressed genes in ESCC tumors vs. normal margin. The fold changes of the gene expression in tumor compared to normal tissue are also defined. Data analyzed by GEsuite GEarray analysis software. The $P < 0.05$ is considered as significant. The q-value, illustrate proportion of false positives observed at a given *P*-value. The smaller q-value means the more significant the differential expression of that gene.

Significant overexpressed genes					Significant underexpressed genes				
Ref seq number	**Symbol**	**T group/ N group**	***P*-value**	**q-value (%)**	**Ref seq number**	**Symbol**	**T group/ N group**	***P*-value**	**q-value (%)**
NM_004068	AP2M1	1.50	0.02	7.18	NM_013230	CD24	0.51	0.03	9.43
NM_004358	CDC25B	4.40	0.00	0	NM_002884	RAP1A	0.60	0.02	9.43
NM_000146	FTL	1.84	0.02	7.18	NM_153815	RASGRF1	0.57	0.00	0
NM_002117	HLA-C	1.59	0.04	8.38	NM_004881	TP53I3	0.25	0.01	0
NM_002127	HLA-G	1.91	0.02	7.18	NM_021141	XRCC5	0.28	0.02	9.43
NM_006597	HSPA8	1.82	0.02	7.18					
NM_000213	ITGB4	2.37	0.03	8.38					
NM_004526	MCM2	2.23	0.05	8.38					
NM_004530	MMP2	1.61	0.01	7.18					
NM_182649	PCNA	2.04	0.00	0					
NM_004223	UBE2L6	1.85	0.05	12.28					

Table 4. Statistical correlations between genes and pathological data.

Gene-gene interaction	Well-differentiated tumors	Stage III	Middle tumors	Lower tumors	T3	Node metastasis	No metastasis
HLA-G/ MMP2	*P*=0.037 r=0.605	—	*P*=0.042 r=0.351	—	*P*=0.018 r=0.343	—	*P*=0.050 r=0.349
HLA-G/ IL10	*P*=0.012 r=0.693	*P*=0.006 r=0.582	—	—	*P*=0.041 r=0.299	*P*=0.020 r=0.473	—
IL10/ MMP2	*P*=0.023 r=0.647	*P*=0.027 r=0.483	—	*P*=0.002 r=0.617	—	—	—

detection control spots on Oligo GEArrays the three efficiently-amplified samples hybridized satisfactorily and could be analyzed by GEsuite GEarray analysis software. Analysis was performed with six images of normal and tumor samples from three subjects (Figure 1). There were 71 genes detected that had 1.5-fold or greater overexpression compared to normal tissue RNA with significant differences in 11 genes (Table 3), while 108 genes showed 1.5-fold or greater underexpression compared to normal tissue RNA with significant differences in 5 genes (Table 3). Table 3 lists the q-values. The smaller q-values show a smaller false detection probability. The rather high q-values in the current study may be a reflection of the low sample size.

The significantly overexpressed genes were AP2M1, CDC25B, FTL, HLA-C, HLA-G, HSPA8, ITGB4, MCM2, MMP2, PCNA, and UBE2L6. Significantly underexpressed genes were CD24, RAP1A, RASGRF1, XRCC5, and TP53I3 ($P \leq 0.05$).

Overexpression of the MMP2 and HLA-G genes and underexpression of XRCC5 and TP53I3 genes were confirmed in three subjects by real-time PCR. MMP2, HLA-G, and XRCC5 markers were chosen to be further validated by real-time PCR in 116 samples from 58 tumors and 58 matched normal tissue margins of Iranian patients with ESCC. We confirmed overexpression of MMP2 and HLA-G and underexpression of XRCC5 with a two-tailed significance level of 0.003 (MMP2), 0.000 (HLA-G), and 0.002 (XRCC5) in the studied population, which identified these genes as possible ESCC tumor markers.

There was a significant, direct relationship between HLA-G ($P = 0.028$) and MMP2 ($P = 0.003$) expression and tumor location. In tumors located in the middle of the esophagus, MMP2 expression correlated with HLA-G ($P = 0.042$), whereas in lower tumors, MMP2 expression correlated with IL-10 ($P = 0.002$). HLA-G expression directly correlated with MMP2 ($P = 0.025$) and IL10 ($P = 0.064$) expression. MMP2 ($P = 0.023$) and HLA-G ($P = 0.012$) expressions correlated with IL-10 in well-differentiated tumors. MMP2 expression also correlated with HLA-G in well-differentiated tumors ($P = 0.037$). In T3 tumors, HLA-G expression correlated with MMP2 ($P = 0.018$) and IL-10 ($P = 0.041$), while in tumors with nodal metastases, HLA-G expression correlated with MMP2 ($P = 0.05$) and IL10 ($P = 0.02$). Correlation results are summarized in Table 4.

A near-significant relationship was observed between tumor invasion and survival ($P = 0.053$); in fact, patients with more invasive tumors (T3 vs. T2) experienced a lower median survival time than those with less invasive tumors (12.0 vs. 27.0 months, respectively; Figure 2).

Discussion

Large numbers of transcriptomic markers loaded on arrays can give comprehensive gene expression profiles of individual carcinomas. Global gene expression profiles are representative of tumor transcriptomes which are the signatures that provide diagnostic, prognostic, and therapeutic information.[35–38] In this study, we have focused on the expression of certain essential genes by using a cancer-specific array. The sample size of the microarray study was low, however, statistical analyses of the results are reliable; data accuracy was validated by the real-time PCR gene expression study. The expression pattern of some of the genes found in our study, such as MMP2, CDC25B, HLA-G, and PCNA, was similar to previous reports; however, the overexpressed genes AP2M1, FTL, UBE2L6, HLA-C and HSPA8 in addition to the underexpressed genes XRCC5, TP53I3, and RAP1A have not been previously reported in ESCC. We analyzed the gene network

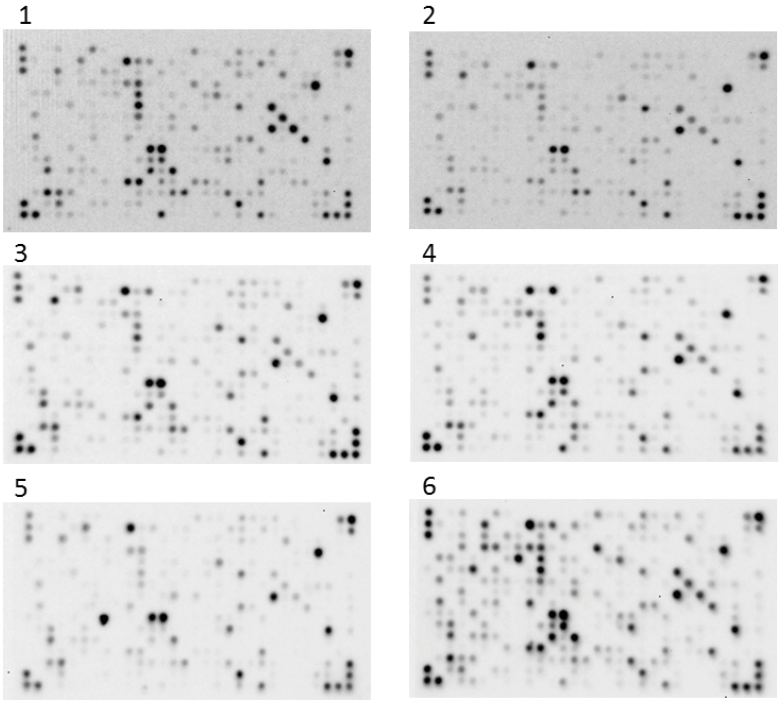

Figure 1. Chemiluminescent images of patients' cRNAs hybridized to Human Cancer GEArrays. Control spots (upper right of the images) were hybridized successfully and confirmed the reliability of hybridization. The images were analyzed by GEarray Expression Analysis Suits (SABiosciences, USA).
1, 3, 5: Normal arrays of 3 patients; 2, 4, 6: Corresponding tumor arrays of patient 1, 2 and 3.

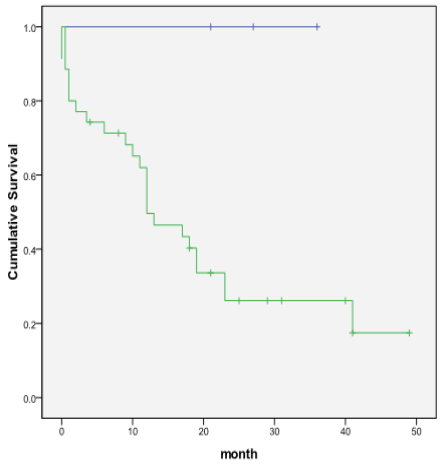

Figure 2. Kaplan-Meier survival curves according to the depth of invasion in TNM staging (Green: T3; Blue*:* T2, *+:* censored data). The survival time difference approximates significance between the T3 and T2 tumors (Log-rank test, *P* = 0.053) as the T3 tumors show a much more aggressive clinical course (Median survival time: 12.0 vs. 27.0 months, respectively).

study in order to categorize all genes with significant expression changes into two main groups: immuno-modulatory response genes that included UBE2L6, AP2M1, HLA-C, HLA-G, HSPA8, and CD24, and those involved in DNA replication and repair that included XRCC5, TP53I3, CDC25B, MCM2, and PCNA. Considering our previous data on immuno-modulatory responses[30] and DNA repair mechanisms involved in ESCC, we chose HLA-G and XRCC5 for validity verification. We also verified MMP2 because it has been previously studied and its overexpression is well-established in ESCC. Therefore, we predicted it would be overexpressed in our samples as well.

Cancer immune surveillance is an important host protection process that inhibits carcinogenesis and preserves cellular homeostasis. Nascent transformed cells can initially be removed by innate immune responses. Evolutionary selection of heterogeneous populations of tumor cells leads to the development of surviving variants that can escape immune attack and allow tumor progression and metastasis.[39] It has been confirmed that malignant transformation of tumors is related to immuno-modulatory elements, such as human leukocyte antigen (HLA).[40] One important factor in immune surveillance is the HLA-G gene product. HLA-G is a non-classical class I antigen, primarily expressed at the materno-fetal interface during pregnancy, where it is thought to protect the fetus from maternal immune recognition. HLA-G expression is restricted in normal tissues; however, its expression exists in a variety of human neoplastic diseases and it could be related to the es-

cape of tumor cells from immune surveillance.[41–43] Many studies have demonstrated overexpression of HLA-G in hepatocellular carcinoma (HCC),[44,45] non-small cell lung cancer (NSCLC),[46–48] breast cancer,[49] endometrial carcinoma,[41] B-cell chronic lymphocytic leukemia (B-CLL),[50] gastric carcinoma, and colorectal cancer.[51,52] The authors of some of these studies have concluded that HLA-G might be a clinical marker useful in diagnosis or prediction of clinical outcomes for malignant diseases. An earlier study indicated that HLA-G expression was associated with poor survival in ESCC patients.[53] Tumor antigen-specific cellular immune response have been shown to play a role in the clinical course of esophageal cancer. HLA-G possibly mediates the interaction between tumor and CD8+ T cells.[40] Our study has shown HLA-G overexpression in 22.4% of subjects. A higher percentage of tumors located in the middle part of the esophagus overexpressed (32.4%) HLA-G compared to those located in the distal part (8.7%). Although this association approximated significance (P = 0.054), we predict a significant association might be observed in a study with a larger sample size.

HLA-G has been introduced as an independent prognostic factor and immunostaining of anti-HLA-G antibodies suggested a role for it as a prognostic indicator for ESCC. HLA-G expression in ESCC tumors significantly correlated with histological grade, nodal involvement, invasion depth, host immune response, and clinical stage of the disease.[53] It was previously shown that HLA-G suppresses the immune response by modulating cytokine secretion and shifting decidual mononuclear cells towards a T helper 2 (Th2) profile. IL-10 is a Th2 cytokine that has been demonstrated to stimulate HLA-G expression, and HLA-G also induces IL-10.[54,55] For this reason, we have analyzed IL-10 as a co-expressed marker for HLA-G using SYBR Green real-time PCR. IL-10 was over-expressed in 51.7% of ESCC patients. There was a significant correlation between HLA-G and IL-10 overexpression in well-differentiated tumors (P = 0.012), stage III tumors (P = 0.006), T3 tumors (P = 0.041), and tumors with node metastases (P = 0.02). Our group previously demonstrated overexpression of IL-10, TGF-beta, and VEGF in ESCC tumors, which indicated immune evasion in ESCC. IL-10 and TGF-beta were overexpressed concomitantly in ESCC tumors.[30] It appears that immuno-modulatory pathways can be suitable subjects for immunotherapy of ESCC. Considering this finding, several studies have successfully introduced dendritic cells for treatment of *in vitro* models of ESCC.[56,57]

Complicated monitoring mechanisms in cells maintain the high-fidelity replication and genome repair. Defects in DNA damage repair processes are essential promoting events in cancer development.[58] Real-time PCR has shown XRCC5 to be underexpressed in 25.5% of subjects in our study (P = 0.002). The XRCC5 protein has previously been reported to be underexpressed in lung squamous cell carcinoma,[31] melanoma, cervical and colon cancers.[59] The protein encoded by XRCC5 gene is the 80 KD subunit of the Ku heterodimer protein, also known as the DNA repair protein. XRCC5 is the DNA-binding component of the DNA-dependent protein kinase. It functions together with the DNA ligase IV-XRCC4 complex in the repair of DNA double-strand breaks by non-homologous end joining and the completion of V(D) J recombination events. Another gene involved in the homologous recombination pathway for double-stranded DNA repair is BRCA2. It is believed that BRCA2 impairment is one of the mechanisms in ESCC carcinogenesis. Because BRCA2 expression analysis is unusual and mutations play an important role in the inactivation of this protein, we did not perform an expression analysis approach for BRCA2. Interestingly, our group has detected familial ESCC in a patient with a BRCA2 mutation who developed sequential breast cancer three years after the development of ESCC (unpublished data). BRCA2 mutations may play a role in esophageal cancer; however, as BRCA2 mutations are reported infrequently in ESCC, BRCA2 mutations may contribute to the development of sequential breast cancer in patients with esophageal cancer. One protein that has shown significant down-regulation is UBE2L6 which belongs to the regulatory network that modulates the function of specific proteins in a cell.[60–64] One probable consequence of underexpression of UBE2L6 could be the augmentation of growth factor receptors[65,66] and an increase in the growth rate of affected cells.[67]

We have shown a correlation between HLA-G and IL-10 with a member of the matrix metalloproteinase (MMP) family, MMP2. Proteins of the MMP family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as disease processes, such as metastasis. This gene encodes an enzyme that degrades type IV collagen, the major structural component of basement membrane. In this study, MMP2 was overexpressed in 39 (67%) of the 58 ESCC patients. Out of 15 dysplasias, 12 (80%) showed positive immunostain results for the MMP-2 protein. Overexpression of MMP-2 in dysplasia as well as ESCC has suggested that these alterations occur in early stages of esophageal tumorigenesis, indicating it might be an early event in esophageal tumorigenesis.[68] In a study performed in China, positive rates of MMP-2 overexpression in the ESCC tumor and corresponding esophageal epithelium tissue were reported as 42.1% and 22.9%, respectively. These rates represented a significant difference between the two groups (c = 4.317, P = 0.038). MMP-2 expression had a significant relationship with tumor invasion depth (P = 0.024), clinical stage (P = 0.035), and lymph node metastasis (c^2 = 4.976, P = 0.026).[69]

In conclusion, our report is the first to analyze expression profiles in ESCC patients in Iran. Genes that significantly up- or down-regulated in ESCC encoded proteins are involved either in immuno-modulatory responses of tumor cells or DNA replication and repair, and subsequent cell cycle regulation in ESCC carcinogenesis pathways. Identification of these tumor-specific biomarkers could prove useful in early diagnoses and treatments for ESCC, leading to improved prognoses and outcomes.

Disclosure statement

All authors confirm that they have no conflict of interest.

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