MEIS1 knockdown may promote differentiation of esophageal squamous carcinoma cell line KYSE-30

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Abstract

Background: MEIS1 (Myeloid ecotropic viral integration site 1), as a homeobox (HOX) transcription factor, has a dual function in different types of cancer. Although numerous roles are proposed for MEIS1 in differentiation, stem cell function, gastrointestinal development and tumorigenesis, the involved molecular mechanisms are poor understood. Our aim in this study was to elucidate the functional correlation between MEIS1, as regulator of differentiation process, and the involved genes in cell differentiation in human esophageal squamous carcinoma (ESC) cell line KYSE-30.

Methods: The KYSE-30 cells were transduced using recombinant retroviral particles containing specific shRNA sequence against MEIS1 to knockdown MEIS1 gene expression. Following RNA extraction and cDNA synthesis, mRNA expression of MEIS1 and the selected genes including TWIST1, EGF, CDX2, and KRT4 was examined using relative comparative real-time PCR.

Results: Retroviral transduction caused a significant underexpression of MEIS1 in GFP-hMEIS1 compared to control GFP cells approximately 5.5-fold. While knockdown of MEIS1 expression caused a significant decrease in EGF and TWIST1 mRNA expression, nearly -8- and -12-fold respectively, it caused a significant increase in mRNA expression of differentiation markers including KRT4 and CDX2, approximately 34- and 1.14-fold, correspondingly.

Conclusion: MEIS1 gene silencing in KYSE-30 cells increased expression of epithelial markers and decreased expression of epithelial-mesenchymal transition (EMT) marker TWIST1. It may highlight the role of MEIS1 in differentiation process of KYSE-30 cells. These results may confirm that MEIS1 silencing promotes differentiation and decreases EMT capability of ESC cell line KYSE-30.

Abbreviations: ALL, Acute lymphoblastic leukemias; bHLH, Basic helix-loop-helix; BMP, Bone morphogenetic protein; ccRCC, Clear cell renal cell carcinoma; CSC, Cancer stem cell; EC, Esophageal cancer; EGF, Epidermal growth factor; ESC, Esophageal squamous carcinoma; ESCC, Esophageal squamous cell carcinoma; GC, Gastric cancer; HSC, Hematopoietic stem cell; ID, Inhibition of differentiation; MEIS1, Myeloid ecotropic viral integration site 1; MPNST, Malignant peripheral nerve sheath tumor; MSC, Mesenchymal stem cell; NSCLC, Non-small-cell lung cancer; SC, Stem cell; TALE, Three amino acid loop extension; TF, Transcriptional factor.

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1 INTRODUCTION

Malignancy is a complex heterogeneous illness introduced through accumulation of different damaging genetic and epigenetic alterations in tumor cells. Notably, disruption of various signaling networks and multiple molecular mechanisms involved in tumor onset and development can lead to extensive deregulation of gene expression profiles in human cancers (Beerenwinkel, Schwarz, Gerstung, & Markowitz, 2014; Chatterjee et al., 2018; Du & Che, 2017). Among identified genetic changes in the cancer etiology, abnormal expression of different gene categories such as tumor suppressors, oncogenes, DNA repair genes, stem cell-related surface markers and cancer stem cells (CSCs) specific transcriptional factors (TFs), can be noted as leading cause of tumorigenesis (Sadikovic, Al-Romaih, Squire, & Zielenska, 2008; Zhao, Li, & Zhang, 2017).

Based on experimental and theoretical data, there are associations between the expression of CSCs markers and cancer-related genes in many tumors. CSCs preserve self-renewal and proliferative potential via inhibiting differentiation signaling pathways during cancer initiation and development (Jin, Jin, & Kim, 2017; Lathia & Liu, 2017). Remarkably, the balance between differentiation and self-renewal capabilities of CSCs produces the bulk of heterogeneous tumor mass contributing in aggressive and stemness phenotypes (Lathia & Liu, 2017). Specific gene expression profiles are needed for tumor cell differentiation which are dictated through different signaling pathways, transcription factor activities, as well as epigenetic alterations such as DNA modifications (Jögi, Vaapil, Johansson, & Pählman, 2012). The involved signaling pathways in CSCs differentiation are BMP (bone morphogenetic protein) and RA (retinoic acid) pathways, while CSCs stemness signaling cascades include JAK/STAT, Wnt/β-catenin, Hedgehog, Notch, PI3K/PTEN, and NF-κB (Jin et al., 2017; Matsui, 2016). The expression profile of differentiation-associated genes is heterogeneous in nearly all types of tumor cells, probably due to the transcriptional activity of a small population of CSCs in combination with numerous partially differentiated cells (Palmer, Schmid, Berger, & Kohane, 2012). Inhibition of differentiation happens through highly expressed ID (Inhibitor of DNA-binding/ differentiation) proteins as regulators of cell fate (Jin et al., 2017).

The most important targets for ID family are basic helix-loop-helix (bHLH) transcription regulators, and homeobox genes encoding DNA-binding domain proteins (Jin et al., 2017; O’Toole et al., 2003).

HOX genes family members, as a subset of homeobox genes, encode TFs with fundamental roles in embryo development and segmentation, as well as differentiation of stem cell (Crist, Roth, Waldman, & Buchberg, 2011; Seifert, Werheid, Knapp, & Tobiasch, 2015). Abnormal expression of HOX genes, often accompanied by DNA hypermethylation, can lead to the developmental diseases and carcinogenesis. The transcribed TFs from HOX genes present two homodomain groups consisting of a conserved 60 amino acids for sequence-specific binding to DNA motifs and a three amino acid loop extension (TALE) (Tsumagari et al., 2013).

MEIS1 (myeloid ecotropic viral integration site 1, OMIM: 601739), as an activator for the HOX members, forms heterodimer complex with HOX transcription factors to recruit either transcriptional co-activator or co-repressor in a DNA sequence-dependent manner, modulating expression of target genes. Numerous TFs including PREP1, HOXA7, HOXA9, and CREB1 regulate MEIS1 expression in different normal tissues and several tumor cells (Torres-Flores, 2013). MEIS1 has an essential role in regulation of stemness state of stem cells, transcription adjustment of self-renewal genes, as well as involved genes in cell development and differentiation, playing an oncogenic role in several tumors (Dardaei et al., 2015; Rad et al., 2016). mRNA and protein expression of MEIS1, as well as its cofactors, were demonstrated in numerous types of malignancies such as leukemia, neuroblastoma, ovarian, renal cell carcinoma, pancreatic, colorectal, gastric, skin, and lung cancers, as well as malignant peripheral nerve sheath tumors (Aksoz, Turan, Albayrak, & Kocabas, 2018). In addition, it has been recently reported that MEIS1 may have cancer stemness property in esophageal squamous cell carcinoma (ESCC) where its downregulation was inversely correlated with stage of progression and metastasis of the tumor (Rad et al., 2016).

Differentiation outcome in squamous epithelium of esophageal needs a serial activity of different specific differentiation-associated genes, and any disruption in this chain may block differentiation process leading to squamous epithelial neoplasia, although the involved molecular mechanisms remain poorly understood (Luo et al., 2014).

Therefore, in the current study, we aimed to assess the impact of MEIS1 gene knockdown on the expression pattern of differentiation-associated genes including TWIST1 (twist family bHLH transcription factor 1, OMIM: 601622), EGF (epidermal growth factor, OMIM: 131530), KRIT4 (Keratin 4, OMIM: 123940), and CDX2 (caudal type homeobox 2,

KEYWORDS

differentiation, ESCC, KYSE-30, MEIS1, TWIST1
OMIM: 600297) in human ESC cell line KYSE-30, to define probable linkage between MEIS1 and differentiation state of the cells.

2 | MATERIALS AND METHODS

2.1 | Cell lines and culture condition

Human ESCC (KYSE-30) and embryonic kidney (HEK293T) cell lines were purchased from the Pasteur Institute Cell Bank of Iran (http://en.pasteur.ac.ir/) and grown in RPMI 1640 medium (Biosera) and Dulbecco’s modified Eagle’s medium (DMEM; Biosera), respectively. Both culture media were supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, USA), 100 U/ml, and 100 μg/ml penicillin-streptomycin (Gibco, USA) at a humidified atmosphere 37°C with 5% CO2.

2.2 | MEIS1 gene expression knockdown

The lentiviral pLKO.1-puro plasmid (Cat. No. SHC003) as a shRNA expression vector was obtained from Sigma-Aldrich (St. Louis, MO). The pLKO.1-puro plasmid DNA was consisted the green fluorescent protein (GFP) gene under the control of the cytomegalovirus (CMV) promoter which express shRNA construct targeting the human MEIS1 (GenBank reference sequence: NM_002398.3). The psPAX2 as a packaging vector and the pMD2.G as a vector encoding the VSV-G (G-protein of the vesicular stomatitis virus) were achieved from Addgene (plasmids 12260 and 12259, respectively, Cambridge, MA). Twenty-one micrograms of pLKO.1-MEIS1 or 21 μg PCDH513b plasmid along with 21 μg of psPAX2 and 10 μg of pMD2.G were transiently cotransfected into HEK293T cells according to the standard calcium phosphate method for producing lentiviral particles. The supernatant containing viral particles was collected at 24 and 48 hr after transfection and filtered through 0.45-μm filter (Orange, Belgium). Then, the supernatant was pelleted using ultracentrifugation (Beckman-Coulter ultracentrifuge XL-100K, USA) at 70,000 x g, 4°C for 1 hr and resuspended in fresh medium. For transduction of KYSE-30 cells, cells were cultured at a density of 1 x 10^5 cells in 6-well plates the day before transduction. On the day of infection, the culture media were replaced with fresh ones containing the lentiviruses for an additional 4–5 days. In order to select the infected cells, the transduced cells were treated with 2 μg/ml puromycin (Invitrogen Corporation, Carlsbad, CA). The transduced KYSE-30 cells with recombinant lentiviral particles of GFP (control) and GFP-shMESI1 were assayed using inverted fluorescence microscopy.

2.3 | RNA extraction, cDNA synthesis, comparative real time PCR, and statistical analysis

Total RNA was isolated from GFP and GFP-shMESI1 transduced ESCC cell line using Tripure reagent (Roche, Nutley, NJ), subsequently DNase I (Thermo Fisher Scientific, Waltham, MA) treatment was performed for preventing DNA contamination. The first strand complementary DNA (cDNA) synthesis was carried out by the oligo-dT method according to the constructor’s procedures (Fermentas, Lithuania). MEIS1 mRNA knockdown was assessed using qRT-PCR. Furthermore, relative comparative changes of KRT4 (GenBank reference sequence: NM_002272.4), CDX2 (GenBank reference sequence: NM_001265.5), EGF (GenBank reference sequence: NM_001963.5), and TWIST1 (GenBank reference sequence: NM_000474.4) mRNA expression were assessed in MEIS1 silenced compared to GFP control cells using a quantitative real-time PCR (SYBR Green, AMPLIQON, Denmark) using gene-specific primer sequences shown in Table 1 on a LightCycler® 96 Real-Time PCR System thermocycler (Roche, Germany). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene was used to normalize data. The 2^ΔΔCt method was applied to measure fold changes of gene expression (Forghanifard, Khales, et al., 2017; Rad et al., 2016). The test was performed triplicate and the thermal profile for MEIS1, KRT4, CDX2,

| Gene   | Forward primer        | Reverse primer        | Annealing T, °C |
|--------|-----------------------|-----------------------|-----------------|
| MEIS1  | ATGACACGGCAGATCTAATCGTTC | TGTCCAAGCCATACACCTTGCT | 62              |
| KRT4   | GCCGTGAGCAGCTCTCTCTTGG | TCCCTCTATGCCTCTCTCTTGCTCAG | 58              |
| CDX2   | ACAGTCGCTACATACACATC  | GATTTCCTCCTCTCTTGGCTC | 55              |
| EGF    | ATGTAGCGGTTGTCTCTTGCTC | ATGGTGTTGGGCTCTTGAGG | 54              |
| TWIST1 | GGAGTCCGCAAGTCTCTACAGG | TCTGGAGGACTGCTGAGG | 57              |
| GAPDH  | GGAAGGTTGAGGGTGGTGCTCG | GTTCAATTGAGGCCAACAATCCACT | 60              |

Note: GenBank reference sequence for the examined mRNA: MEIS: NM_002398.3, KRT4: NM_002272.4, CDX2: NM_001265.5, EGF: NM_001963.5, and TWIST1: NM_000474.4.
EGF, and TWIST1 included an initial denaturation at 95°C for 10 min, followed by 45 cycles 94°C (30 s), specific annealing temperature (30 s), and 72°C (30 s).

The SPSS 19.9 statistical package (SPSS, Chicago, IL, USA) was applied for statistical data analysis. p value < 0.05 was regarded as statistically significant. The χ² or Fisher exact tests, as well as Pearson's correlation were used to evaluate the association between gene expressions.

3 | RESULTS

3.1 | Downregulation of MEIS1 in ESCC cell line KYSE-30

After lentiviral-mediated MEIS1 knockdown, the expression of MEIS1 was evaluated in GFP-hMEIS1 in comparison with pCDH513b GFP-control KYSE-30 transduced cells (>95% positive) to confirm MEIS1 silencing. The fluorescent microscopy images of transduced GFP-shMESI1 and GFP control KYSE30 cells are shown in Figure 1. The significant underexpression nearly 5.5 (log2 fold change) of MEIS1 was detected in lentiviral GFP-hMESI1 transduced cells compared to GFP control.

3.2 | Knockdown of MEIS1 changed the expression of differentiation genes

Having confirmed the MEIS1 gene silencing in KYSE-30 cells, we analyzed expression of specific epithelial and epithelial-mesenchymal transition markers in examined cells. Downregulation of MEIS1 led to a significant decrease in the levels of EGF and TWIST1 mRNA expression (-8- and -12-fold, respectively) in GFP-hMESI1 compared to control cells. Additionally, MEIS1 underexpression significantly increased expression of KRT4 and CDX2 mRNA levels with 34- and 1.14-fold, respectively. The results are summarized in Figure 2.

4 | DISCUSSION

Different biological processes are involved in tumorigenesis such as cell proliferation, apoptosis, differentiation, metastasis, vascularization, as well as self-renewal. In addition, deregulation of signaling pathways can induce tumorigenesis and cancer progression. Accordingly, identification of the involved genes in tumor emergence and understanding of underlying molecular mechanisms are required for representing
and CDX2 mRNA expression, respectively. KRT4, –12-, –8-fold decrease and 34-, 1.14-fold increase in TWIST1, EGF, hTMEIS1 nearly 5.5-fold compared to GFP control cells causing a MEIS1 cells. Retroviral transduction silenced expression in GFP- on TWIST1, EGF mRNA expression in KYSE-30 cells including TFs, miRNAs, and cellular metabolites (Aksoz et al., 2018). In addition, MEIS1 involves in stem cell growth and self-renewal, as well as cell development and differentiation during embryogenesis (Zhu et al., 2017). Moreover, it plays a critical role in tumor cells approximately exhibit markers of differentiated epithelial cells including KRT4 and CDX2, while a significant down-regulation was observed in EGF and TWIST1 gene expression, as specific markers of cell proliferation and EMT, respectively. These results may highlight the critical role of MEIS1 in regulation of cell differentiation through modulation of gene expression pattern in ESC cell line KYSE-30.

Homeobox genes function as master transcriptional regulator of stem cell (SC) differentiation from embryonic stages to adult tissues, and their aberrant expression is associated with tumorigenesis (Rodrigues, Esteves, Xavier, & Nunes, 2016). (Grier et al., 2005). Among homeobox genes, MEIS1 involves in stem cell growth and self-renewal, as well as cell development and differentiation during embryogenesis (Zhu et al., 2017). Moreover, it plays a critical role in tumor progression, and functions as a negative regulator of cell cycle checkpoints, as well as cell proliferation and apoptosis in some malignancies such as prostate, non-small-cell lung cancer (NSCLC), clear cell renal cell carcinoma (ccRCC), and ESCC (Aksoz et al., 2018; Crist et al., 2011).

Intriguingly, numerous reports have demonstrated that different molecular modulators are related to MEIS1 expression including TFs, miRNAs, and cellular metabolites (Aksoz et al., 2018). In addition, MEIS1 can modulate expression of involved genes in cell differentiation and proliferation such as HIF1/2, GATA1, CCND1/3, SOX3, and PBX1 (Torres-Flores, 2013). It has been confirmed that MEIS1 is involved in progression of different malignancies. It functions as a double-edged sword with a dual function (either oncogenic or tumor suppressive role) in diverse cell types depending on the cell context. Oncogenic role of MEIS1 transcription factor is detected in a variety of malignancies including leukemia, malignant peripheral nerve sheath tumors (MPNST), nephroblastoma, and ovarian, where it promotes cell proliferation and inhibits programmed cell death (Blasi, Bruckmann, Penkov, & Dardaei, 2017). On the other hand, several studies have demonstrated that MEIS1 acts as potential tumor suppressor in some tumors such as ccRCC, prostate, lung, gastric, and colorectal cancers through promoting cell differentiation and inhibition of epithelial cell proliferation (Chen et al., 2012; Song, Wang, & Wang, 2017; Zhu et al., 2017).

MEIS1 silencing through RNAi mechanism was correlated with epithelial cancer cells (NSCLC) proliferation and accelerated cell cycle progression in vivo (Li, Huang, Guo, & Cui, 2014). Moreover, MEIS1 knockdown inhibited DNA replication in acute lymphoblastic leukemias (ALL) through regulation of involved genes in cell cycle process (Orlovsky et al., 2011). On the other hand, MEIS1 ectopic expression in gastric cancer (GC) cells not only suppressed critical cancer cell properties including cell proliferation, colony formation, anchorage independent growth, epithelial mesenchymal transition (EMT), migration, and invasion, but also induced apoptosis and cell cycle arrest at G1/S transition in vitro (Song et al., 2017). In addition, an inverse association between MEIS1 and SRY (sex determining region Y)-box 2 (SOX2) in ESCC tumor samples was reported (Rad et al., 2016). MEIS1 silencing in ESC cell line KYSE-30 has resulted in overexpression of SOX2 as a stemness factor. Such results may proposed suppressive role of MEIS1 on SOX2 gene expression in ESCC to inhibit stemness state progression (Rad et al., 2016). It has been illustrated that MEIS1 silencing in mouse embryonic carcinoma suppressed differentiation in neural cells, while its ectopic expression induced differentiation via expression of neural progenitor markers including GLAST, BLBP, SOX1, and Nestin (Yamada, Urano-Tashiro, Tanaka, Akiyama, & Tashiro, 2013). Consequently, induced OCT4 can increase MEIS1 expression and the upregulated MEIS1 can repress OCT4 expression, as a main gene of pluripotency, in a negative feedback loop (Yamada et al., 2013). Thus, modulation of OCT4 and SOX2 protein expression occur via differentiation signals, and MEIS1 is contributed in this modulation of tumor cell differentiation (Rad et al., 2016; Yamada et al., 2013).

During differentiation process, expression of differentiation markers is increased, while expression of Yamanaka factors (OCT4, SOX2, KLF4, and MYC), which are activated in embryonic stem cells, is decreased. In addition, high-level expression of Yamanaka factors can alter the gene expression pattern of the cell from differentiated to de-differentiated state that lead to the cell reprogramming (Miyamoto, Furusawa, & Kaneko, 2015). Since tumor cells approximately exhibit markers and properties of embryonic stem cells, low level of OCT4...
or SOX2 is necessity for supporting MEIS1 expression to promote the maintenance of differentiation in such cells (Tucker et al., 2010).

Here we have sought to investigate the significant changes in expression level of some gene related to tumor cell differentiation following MEIS1 silencing in ESC cell line KYSE-30. MEIS1 silencing resulted in suppression of the involved genes in cell proliferation (EGF) and EMT (TWIST1), leading to tumor cell differentiation in ESC cell line KYSE-30.

TWIST1, as a bHLH transcription factor, is a key regulator of different cellular processes. It identify E-box consensus sequence in promoter of target genes and adjust downstream gene expression (Izadpanah, Abbaszadegan, Fahim, & Forghanifard, 2017). It was revealed in this study that MEIS1 knockdown causes a significant decrease in TWIST1 gene expression in KYSE-30 cells. TWIST1 not only involves in embryonic organogenesis, specification, and differentiation, but also is associated with tumor initiation, angiogenesis, stemness and EMT (epithelial-mesenchymal transition) promotion, leading tumor cell invasion and metastasis in a variety of human malignancies (Forghanifard, Rad, et al., 2017). It has been indicated that silencing of TWIST1 lead to increase osteoblast differentiation in mesenchymal stem cells (MSCs) by upregulation of the involved genes in FGF/ERK and BMP signaling pathways (Miraoui, Severe, Vaudin, Pagès, & Marie, 2010). Upregulation of numerous Zn-finger TFs such as SNAIL1/2, ZEB1/2, and TWIST1/2 that involve in several cell signaling pathways can lead to loss of E-cadherin, the hallmark of EMT progression (Cheng, Auersperg, & Leung, 2012; Forghanifard, Khales, et al., 2017). TWIST1 ectopic expression leads to downregulation of E-cadherin and activation of mesenchymal markers. Inverse correlation between upregulation of TWIST1 and decreased expression of E-cadherin has been shown in several malignancies (Sasaki et al., 2009). According to the role of TWIST1 in EMT, its significant decreased expression following MEIS1 silencing in KYSE-30 cells may inhibit EMT progress and invasiveness behavior of the cells, and reverse the process of mesenchymal transition which may result in tumor cell differentiation.

EGF (epidermal growth factor), as a tyrosine kinase ligand, stimulates various cellular responses such as epithelial cells differentiation and proliferation, apoptosis, migration, as well as cell division and survival (Li, Shan, et al., 2014). Interaction between EGF and its receptor (EGFR or ErbB-1) leads to activate growth factor-mediated intracellular downstream pathways such as PI3K/AKT and RAS/MEK/ERK that result in EGF-induced EMT (Bodnar, 2013). In this study, silencing of MEIS1 expression significantly suppressed the EGF expression in KYSE-30 cells indicating that MEIS1 is involved in the EGF related signaling cascades. It is suggested that downregulation of EGF, as an epithelial factor, leads to decrease EMT (Li, Shan, et al., 2014). Taken together, our results illustrate that underexpression of TWIST1 and EGF, as two prevalent TFs of EMT promotion, can lead to a suppressed EMT in KYSE-30 cells as an invasive cell line.

We have found that stable MEIS1 knockdown induces KRT4 and CDX2 upregulation, indicating these genes are involved in KYSE-30 differentiation. CDX2, as a ParaHox family of homeobox genes, has a key functions in intestinal epithelial differentiation, proliferation, maintenance of the intestinal phenotype and regulation of intestine specific gene transcription program, WNT-mediated beta-catenin signaling as well as tumorigenesis (Dong & Guo, 2015). In addition, downregulation of CDX2 leads to development of intestinal neoplasia and is introduced as a prognostic marker for colon cancer (Dong & Guo, 2015). Ectopic expression of the intestine-specific homeobox transcription factor CDX2 cause Barrett’s esophagus and gastric-intestinal metaplasia (Joo, Park, & Chun, 2016). Furthermore, loss of CDX2 expression was found in various ESC cell lines due to promoter hypermethylation (Guo et al., 2007). In line with these reports, our results also confirmed this pattern of the gene expression. While the EMT involving genes were downregulated and EMT process was suppressed, the epithelial markers were upregulated to fix epithelial state of the cells inhibiting mesenchymal converting. Since all these changes in gene expression pattern was induced by MEIS1 knockdown, it may be hypothesized that MEIS1 promotes EMT and suppresses cell differentiation in ESC cell line KYSE-30.

Cytokeratins (CKs), as intermediate filament cytoskeletal proteins, are the major components of normal epithelium and squamous tumor tissues which are expressed in different grades of cell differentiation and introduced as indicator for predicting of tumor progression in ESCC (Cintorino et al., 2001; Singh et al., 2009). KRT4, as a member of intermediate filament proteins family, is expressed in suprabasal layers of nonkeratinizing stratified epithelium such as esophagus and regulated in a differentiation-dependent manner (Alam, Sehgal, Kundu, Dalal, & Vaidya, 2011). Interestingly, expression level of KRT4 protein was decreased in transition from normal esophageal epithelium to invasive tumor of stratified squamous epithelium and associated with cancer progression (Chung et al., 2006). Therefore, the increased expression of KRT4 after MEIS1 silencing may suggest the putative correlation of KRT4 overexpression with differentiation state of the KYSE-30 cells. Following stable MEIS1 gene silencing, we have found a significant increase in CDX2 and KRT4 mRNA expression in KYSE-30 cells, which may probably orient the cells toward differentiation phenotype. Altogether, these results correlate MEIS1 expression and the involved genes in maintenance of tumor cell differentiation, introducing MEIS1 as a probable key regulator in this process in ESC cells, and presenting a potentially molecular mechanism for regulation differentiation and EMT processes in ESC cell line KYSE-30.

In conclusion, we showed that MEIS1 is significantly correlated with the involved genes in cell differentiation and EMT processes in KYSE-30 cells. Having confirmed the correlation of MEIS1 with TWIST1 and EGF, as well as its inverse
association with epithelial cell markers including KRT4 and CDX2, we may propose a role for MEIS1 in progress of KYSE-30 cell dedifferentiation. These findings may suggest that MEIS1 gene repression can be a therapeutic strategy to inverse invasive characteristic of the ESC cells. To the best of our knowledge, this is the first report revealing regulatory role of MEIS1 on expression of the involved genes in EMT and differentiation in esophageal squamous carcinoma cell line KYSE-30.

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**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

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