RESEARCH ARTICLE

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HMGA2 Overexpression Is Associated with Poor Prognosis and EGFR Signaling Activation in Oral Squamous Cell Carcinoma

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Abstract

Objective: To evaluate the clinical significance of *HMGA2* expression and its association with oncogenic signaling pathways in oral squamous cell carcinoma (OSCC). **Methods:** RNA-seq data and clinical information of OSCC patients (n=253) and normal oral tissues (n=27) were obtained from The Cancer Genome Atlas (TCGA). *HMGA2* expression was compared between normal oral tissues and OSCC tissues, and patients were stratified into *HMGA2*-high and *HMGA2*-low groups based on median expression. Differentially expressed genes were identified using DESeq2, followed by pathway enrichment analyses using Reactome and WikiPathways. Functional validation was independently performed in two OSCC cell lines using siRNA-mediated *HMGA2* knockdown. Cell proliferation, colony formation, and migration were assessed, and EGFR signaling and EMT-related proteins were analyzed by Western blotting. **Results:** *HMGA2* expression was significantly higher in OSCC tissues than in normal oral tissues (adjusted $p < 0.001$) and was associated with advanced AJCC stage and poorer overall survival ($p < 0.05$). Pathway analyses revealed significant enrichment of EGFR-related signaling and epithelial–mesenchymal transition (EMT)-associated processes in the *HMGA2*-high group. *HMGA2* knockdown significantly reduced proliferation, clonogenicity, and migration of OSCC cells, accompanied by decreased expression of mesenchymal markers and reduced activation of EGFR and its downstream effectors, including *AKT, ERK*, and *STAT3. EGFR* pathway activity was partially restored by exogenous EGF treatment. **Conclusion:** *HMGA2* overexpression is associated with poor prognosis in OSCC and functions as both a prognostic biomarker and a functional contributor to aggressive tumor behavior through EGFR-associated signaling and EMT-related phenotypes.

Keywords: Oral squamous cell carcinoma- EGFR signaling- EMT- Biomarker- Therapeutic target

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Introduction

Oral squamous cell carcinoma (OSCC), accounting for over 90% of oral cancers, ranks among the six most common cancers worldwide [1]. Despite advances in surgery, radiotherapy, chemotherapy, and the introduction of targeted and immunotherapies, the overall 5-year survival rate remains below 50%, largely due to late diagnosis and treatment resistance [2, 3]. Major risk factors include tobacco use, alcohol consumption, and human papillomavirus infection [4]. Notably, approximately 26% of OSCC cases recur after curative treatment, and more than 80% of these recurrences occur within the first two years, underscoring the urgent need for improved prognostic biomarkers and novel therapeutic targets [5].

EGFR signaling is frequently activated in OSCC, and its limited therapeutic efficacy in clinical settings highlights the need to better understand upstream regulatory mechanisms [6]. Although several EGFR-targeted therapies have been evaluated, their inconsistent clinical benefit suggests that key upstream modulators governing EGFR activation and signaling output remain insufficiently characterized in OSCC. Identifying such regulators may provide critical insight into both disease progression and treatment resistance, thereby offering opportunities for improved prognostic stratification and therapeutic intervention.

HMGA2 is a chromatin-associated protein that is highly expressed during embryonic development but largely silenced in adult tissues [7]. It regulates transcription by binding to AT-rich DNA regions and modifying chromatin structure [8], and recent studies further suggest that it can directly condense nucleosomes to control gene accessibility [9]. Such findings point to broader roles for *HMGA2*, including chromatin condensation and phase separation, which may provide additional layers of oncogenic regulation.

Aberrant re-expression of *HMGA2* has been reported in multiple cancers, including breast, lung, prostate, and hematologic malignancies, where it is linked to enhanced proliferation, invasion, and poor clinical outcomes [10-12]. In OSCC, however, the clinical relevance of *HMGA2*

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expression and its functional positioning within oncogenic signaling networks are not yet fully charaterized. In particular, the extent to which *HMGA2* quantitatively influences EGFR pathway activation and downstream signaling output in OSCC has not been systematically examined. Despite reported bioinformatic associations, it remains unclear whether *HMGA2* directly modulates EGFR signaling activity in OSCC or merely reflects a correlated transcriptional state. Moreover, prior studies have rarely integrated patient-derived transcriptomic data with mechanistic in vitro validation to define the functional hierarchy between *HMGA2* and EGFR signaling in OSCC. Therefore, in this study, we integrate TCGA-based analyses with targeted loss-of-function experiments to determine whether *HMGA2* functionally contributes to EGFR pathway activation in OSCC. Using transcriptomic data from The Cancer Genome Atlas (TCGA), we found that *HMGA2* is significantly upregulated in OSCC, with high expression associated with advanced tumor stage and poor prognosis. Consistent with our findings, TCGA-based analyses in other malignancies have identified *HMGA2* as implicated in tumor progression [13]. Despite these existing bioinformatic associations, the functional link between *HMGA2* and the EGFR signaling cascade, specifically within the unique context of OSCC, remains largely under-explored experimentally. Accordingly, this study combines TCGA analysis with comprehensive in vitro validation to elucidate the causal role of *HMGA2* in OSCC progression.

Functional assays further demonstrated that *HMGA2* promotes proliferation, migration, and epithelial–mesenchymal transition, in part by sustaining EGFR signaling and its downstream pathways, including PI3K/AKT, MEK/ERK, and STAT3. Importantly, depletion of *HMGA2* reduced activation of these oncogenic cascades, and exogenous EGF treatment partially restored signaling, supporting a mechanistic link between *HMGA2* and EGFR pathway activity.

These findings suggest *HMGA2* as both a prognostic biomarker and a potential therapeutic target in OSCC, providing a basis for future studies for biomarker-guided strategies and novel treatment approaches to overcome resistance in this aggressive cancer.

Materials and Methods

Differentially Expressed Gene Identification in TCGA Database

RNA-seq expression profiles and corresponding clinical data of patients with oral squamous cell carcinoma (OSCC; n=253) and normal oral tissues (n=27) were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov). Patients with incomplete expression or clinical data were excluded. Based on the median expression value of *HMGA2*, patients were divided into high-expression and low-expression groups. Differentially expressed genes (DEGs) between the two groups were identified using the DESeq2 package in R software (https://cran.r-project.org). Genes with |log2Fold Change| > 1 and an adjusted p-value (FDR) < 0.1, based on the Benjamini–Hochberg method, were considered statistically significant. Volcano plots were generated using the ggplot2 package to visualize overall differential expression patterns.

Functional and Pathway Enrichment Analyses of DEG

Functional enrichment analyses were conducted to explore the biological significance of *HMGA2*-associated DEGs. Reactome pathway enrichment was performed using the ReactomePA R package, and WikiPathways enrichment was conducted via the enrichR interface. Enrichment scores and adjusted p-values were used to rank the significance of pathways, and the top-ranked pathways were visualized using bar plots. Pathway–gene interaction networks were further generated to illustrate the functional associations between DEGs and enriched signaling pathways. Pathway enrichment significance was determined using p-values<0.05

Pathway–Gene Network Visualization

Functional association networks were constructed based on enrichment results and differentially expressed genes. The resulting interaction data were then visualized and analyzed using the Cytoscape software platform. Both Reactome and WikiPathways-derived networks were analyzed to offer complementary insights into the *HMGA2*-related oncogenic mechanisms.

Cell Lines and Materials

The immortalized normal human fibroblast cell line (hFBs) and the OSCC cell lines SCC15, SCC25, HEP2, and YD8 were used in this study. This study focused on HPV-negative OSCC, which represents the majority of oral cavity cancers. SCC15 and SCC25 cells were cultured in DMEM/F12 (Gibco, Grand Island, NY, USA; catalog number: C11330500BT). HEP-2 cells were cultured in EMEM (ATCC, Manassas, VA, USA). YD8 cells were cultured in RPMI-1640 (ATCC, Manassas, VA, USA). All the cell culture media was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco). Cells were grown in a cell culture incubator (37 ◦C with 5% CO2 under humidified conditions).

Western Blot Analysis

Each sample was lysed in buffer containing 0.1% sodium dodecyl sulfate, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 50 mM Tris, pH 8.0, 150 mM NaCl, and a protease inhibitor (Roche Applied Science, Vienna, Austria, pH 7.4). The primary antibodies involved included anti-*HMGA2*, anti-EGFR, anti-phospho-EGFR, anti-ERK, anti-phospho-ERK, anti-AKT, anti-phospho-AKT, anti-STAT3, anti-phospho-STAT3, anti-E-cadherin, anti-N-cadherin, anti-SLUG, anti-VIMENTIN and anti-β-actin (1:1000; Cell Signaling Technology Inc., Danvers, MA, USA). After incubation with the corresponding horseradish peroxidase-conjugated secondary antibodies (1:5000; Santa Cruz Biotechnology), immunoreactive bands were visualized by enhanced chemiluminescence detection (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Densitometric analysis was performed using ImageJ software. Target protein expression levels were normalized to β-actin, and phosphorylated protein

levels were further normalized to their corresponding total protein levels. All western blot experiments were independently repeated at least three times (biological replicates).

Small-Interfering RNA (siRNA) Transfection

Transient transfection was performed once cells reached 60% confluence using Lipofectamine RNAiMAX reagent (Invitrogen) for siRNA following the manufacturers' standard protocols. The siRNA for *HMGA2* (seq no. sense, 5′-CAGCAATCTGTCGCTAAGG dTdT-3′; antisense, 5′-CCTTAGCGACAGATTGCTG dTdT-3′) and the siRNA for the control group were acquired from Invitrogen. All experiments were repeated at least three times.

Cell Proliferation Assay

In total, 6×10^3 cells per well in 96-well plates were seeded in media. Cell viability was then measured using WST-1 (Roche Diagnostics, Indianapolis, IN, USA). Background absorbance from cell-free wells was subtracted for normalization, and the conversion of WST-1 to formazan was quantitated at 450 nm using an enzyme-linked immunosorbent assay reader. Results were normalized to the control group and expressed as relative percentages.

Cell Migration

Transwell membranes (24-well; Costar, Cambridge, MA, USA) were coated without Matrigel for the migration assay. In total, 1×10^5 cells in serum-free medium were seeded onto the upper chamber, and 750 μL of medium supplemented with 10% FBS was added to the lower chamber. After incubation for 24 h (for migration), the cells adhering to the upper surface of the membrane were removed with a cotton swab. The migrated cells that adhered to the lower surface were stained with crystal violet and counted in four representative fields using light microscopy (×40 magnification).

Clonogenic Assay

Cells were seeded in 6-well plates at 5×10^3 cells/well in 2 mL of medium. The medium was replaced with new medium every three days, and the cells allowed to grow for 10 days. The colonies were fixed in 4% formaldehyde, stained with crystal violet, and then imaged and counted.

Statistical Analysis

All in vitro experiments were repeated three times, and data are presented as the mean ± standard deviation. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied, whereas pairwise comparisons were performed using Student's t-test. A p-value < 0.05 was considered to indicate statistical significance. The cohort was stratified into *HMGA2*-high and *HMGA2*-low subgroups based on the median *HMGA2* expression. Differential gene expression between these subgroups was assessed using the DESeq2 package, with results adjusted for multiple comparisons via the Benjamini–Hochberg procedure. Genes meeting the threshold of adjusted p-value < 0.05 and $|\log2FC| > 1$ were classified as significantly differentially expressed.

Results

HMGA2 is overexpressed and associated with tumor progression in OSCC

As shown in Figure 1A, *HMGA2* expression levels were higher in the OSCC samples than in normal tissue in the TCGA database. Next, we evaluated the correlations between *HMGA2* expression and clinicopathological factors affecting the prognoses of OSCC patients. As shown in Figure 1B, advanced AJCC stage was significantly associated with high *HMGA2* expression. To explore whether *HMGA2* plays a critical role in OSCC cell lines, the expression of *HMGA2* protein at the protein level was examined in the normal cell line (hFB) and in 4 OSCC cell lines (SCC15, SCC25, YD8, HEP-2). The majority of the OSCC cell lines showed significantly higher *HMGA2* protein expression at the protein level (Figure 1C). We next evaluated the correlations between *HMGA2* expression and overall survival in OSCC patients. Patients with high *HMGA2* expression had poorer survival rate than those low expression (Figure 1D).

Association of HMGA2 with EGFR-related signaling pathways and EMT in OSCC

Using TCGA OSCC cohort, patients were stratified into *HMGA2*-high and *HMGA2*-low groups based on median expression levels. Patients with high *HMGA2* expression exhibited markedly different molecular characteristics compared with the low-expression group. The volcano plot illustrated a pronounced contrast in gene expression, with a large number of genes being significantly upregulated (red) or downregulated (blue) in patients with high *HMGA2* expression compared to those with low expression (Figure 2A). To further elucidate the biological functions of *HMGA2*-related DEGs, pathway enrichment analyses were performed.

Reactome pathway analysis (Figure 2B) provided further evidence supporting the oncogenic role of *HMGA2*. The enriched pathways were strongly associated with cell adhesion and junction remodeling, including tight junction and cell–cell junction organization, which are critical hallmarks of epithelial–mesenchymal transition (EMT) and tumor invasiveness. Moreover, transcriptional regulation by the AP-2/TFAP2 family and inflammatory signaling (IL-10, pro-inflammatory response) [14] were significantly enriched, suggesting that *HMGA2* may facilitate a pro-tumor microenvironment. Importantly, enrichment of MET–PTK2 activation and MET-driven motility pathways, together with EGFR-related signaling, underscored the role of *HMGA2* in driving tumor cell survival, migration, and metastatic progression.

Wiki Pathways analysis (Figure 2C) further substantiated the oncogenic role of *HMGA2* in OSCC. Significantly enriched pathways prominently featured COX2/EGFR, PI3K–AKT–mTOR, and NF-κB/HIF1A survival signaling all well-established drivers of tumor progression and therapy resistance.

Notably, multiple pathways associated with EMT,

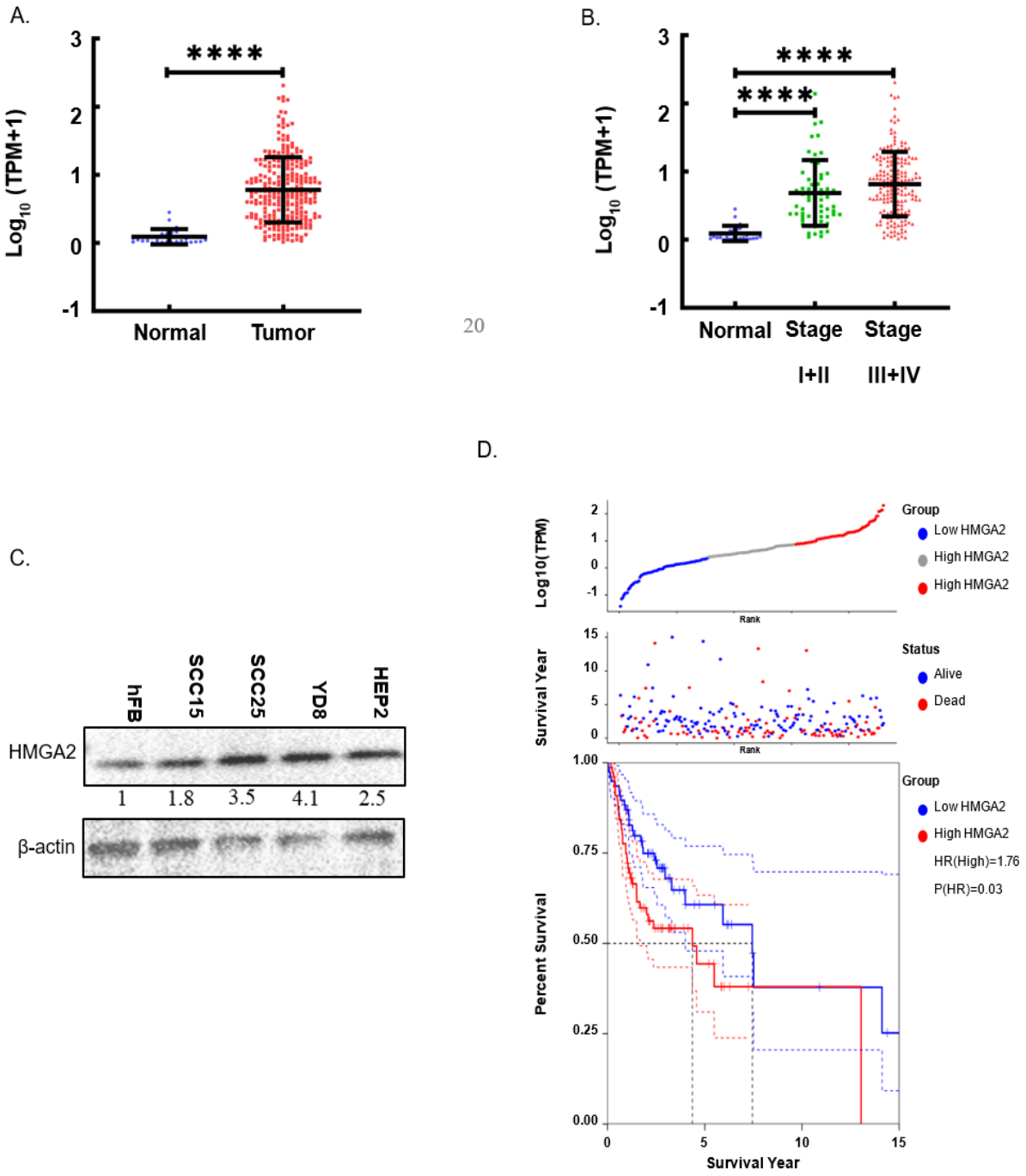

Figure 1. *HMGA2* is upregulated in OSCC and is associated with tumor stage and poor overall survival. A. HMGA2 expression levels in OSCC tissues compared with normal tissues in the TCGA database. B. Correlation of *HMGA2* expression with AJCC stage in OSCC patients. C. Western blot analysis of HMGA2 protein expression in a normal fibroblast cell line (hFB) and four OSCC cell lines (SCC15, SCC25, HEP-2, YD8). These results are representative of three independent experiments. D. Kaplan–Meier survival curves showing overall survival of OSCC patients stratified by high versus low *HMGA2* expression. Statistical significance for comparisons among AJCC stages was determined by one-way ANOVA followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.0001$ (****).

including TGF-β receptor signaling and EMT-related transcriptional programs, were also markedly enriched, underscoring the link between *HMGA2* expression and enhanced invasive potential. Collectively, these results strongly support a model in which *HMGA2* overexpression drives oncogenesis in OSCC by activating EGFR-associated signaling cascades, potentiating EMT, and fostering a pro-survival, inflammatory tumor microenvironment. To further delineate the functional networks associated with *HMGA2* expression, we constructed a pathway–gene interaction map based on DEGs between *HMGA2*-high and *HMGA2*-low OSCC patients (Figure 2D). The network highlighted multiple cancer-related pathways, including extracellular matrix degradation, cell adhesion, MET–PTK2 signaling, GPCR downstream signaling, and transcriptional regulation by

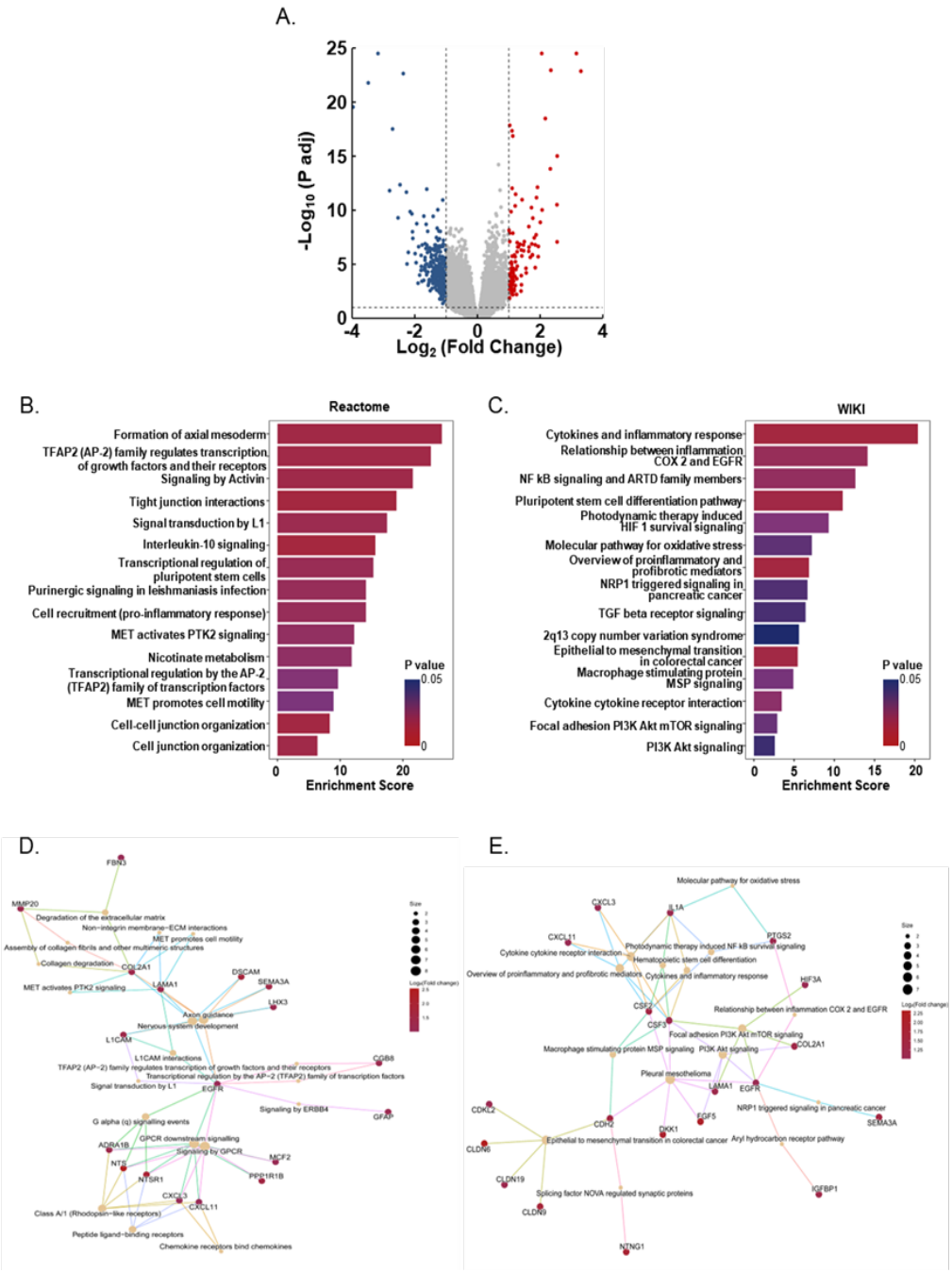

Figure 2. *HMGA2* is associated with EGFR Signaling and EMT-related Pathways in OSCC A. Volcano plot of differentially expressed genes (DEGs) between HMGA2-high and HMGA2-low OSCC patients in the TCGA cohort. B. Reactome pathway enrichment analysis of HMGA2-related DEGs, showing enrichment in cell adhesion, junction remodeling, EMT, and EGFR-associated signaling. C. WikiPathways enrichment analysis highlighting COX2/EGFR, PI3K–AKT–mTOR, NF-κB/HIF1, and TGF-β receptor signaling pathways linked to HMGA2 overexpression. D. Pathway–gene interaction network based on DEGs, revealing EGFR as a central hub connecting multiple oncogenic pathways. E. Pathway–gene interaction network from WikiPathways analysis emphasizing EGFR-centered signaling, EMT-related programs, and inflammatory responses.

the AP-2/TFAP2 family. Notably, the results converged on EGFR as a central hub gene, connecting several oncogenic pathways such as ERBB4 and EGFR signaling as well as cell adhesion-related programs. This indicates that EGFR may serve as a mediator linking *HMGA2* overexpression to downstream oncogenic signaling.

Importantly, many of the enriched pathways were directly or indirectly associated with EMT, extracellular matrix remodeling, and chemokine signaling, which likely contribute to tumor invasiveness. Together, these findings suggest that *HMGA2* overexpression promotes OSCC progression through an EGFR-centered regulatory network, thereby facilitating EMT, cell motility, and pro-tumor microenvironment remodeling.

The pathway–gene interaction network based on WikiPathways analysis (Figure 2E) further emphasized the oncogenic role of *HMGA2*. The network revealed strong enrichment in COX2/EGFR signaling, PI3K–AKT–mTOR pathway, NF-κB/HIF1A survival signaling, and cytokine–cytokine receptor interactions, highlighting

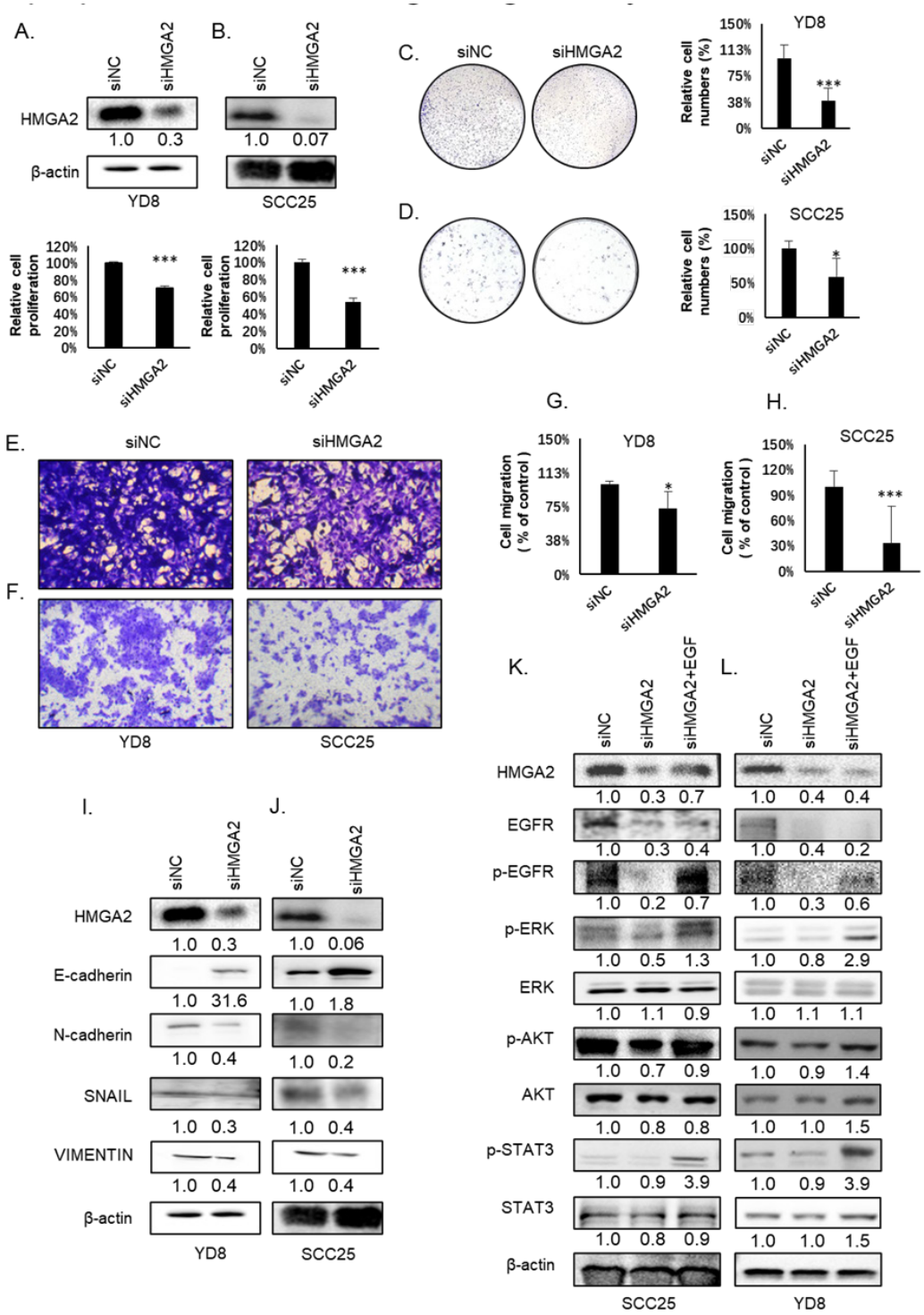

Figure 3. HMGA2 knockdown attenuates tumorigenic properties and EGFR signaling activity in OSCC. A, B. Cell proliferation assessed by WST-1 assay in SCC25 and YD8 cells after HMGA2 knockdown. B, D. Colony formation capacity of SCC25 and YD8 cells following HMGA2 depletion. E-H. Migration of SCC25 and YD8 cells upon HMGA2 silencing compared with controls. I, J. Expression of EMT markers analyzed by Western blot in SCC25 and YD8 cells after HMGA2 knockdown. K, L. EGFR signaling activity examined by Western blot showing changes in EGFR and downstream effectors, with rescue by exogenous EGF treatment. All data are presented as the mean ± standard deviation of three independent experiments. Statistical analysis for multiple-group comparisons was performed using one-way ANOVA followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$ (*) and $p < 0.001$ (***).

the convergence of inflammatory and survival pathways. Notably, EMT-related programs, including epithelial–mesenchymal transition in colorectal cancer and extracellular matrix remodeling, were closely connected to hub genes within the network.

These results indicate that *HMGA2* overexpression promotes OSCC progression through activation of EGFR-centered oncogenic signaling, while simultaneously enhancing EMT processes and pro-inflammatory responses that facilitate tumor invasion and immune

evasion.

HMGA2 knockdown attenuates tumorigenic properties and is associated with reduced EGFR signaling in OSCC

HMGA2 expression was knocked down using siRNA in SCC25 and YD8 cells to evaluate its impact on cellular phenotypes associated with OSCC malignancy. Cell proliferation was assessed using the WST-1 assay, and *HMGA2* depletion markedly reduced the growth of YD8 and SCC25 cells (Figure 3A, B). In addition, the effects of *HMGA2* knockdown on clonogenic potential were evaluated, revealing a significant reduction in colony formation in both YD8 and SCC25 cells (Figure 3C, D). Subsequent analyses evaluated the impact of *HMGA2* silencing on tumor cell motility. Depletion of *HMGA2* significantly impaired the migration of YD8 and SCC25 cells relative to controls (Figure 3E-H). A widely recognized association exists between EMT and the invasive behavior of cancer cells. Pathway enrichment analysis revealed that *HMGA2*-associated DEGs were significantly enriched in cell adhesion and junction organization pathways (including tight junctions and cell–cell junction remodeling), suggesting a potential role of *HMGA2* in modulating cellular adhesion properties (Figure 2B, C). Pearson correlation analyses were performed to assess the relationships between *HMGA2* and EGFR, MAPK1, AKT1, STAT3, and representative epithelial and mesenchymal markers based on TCGA transcriptomic data (Supplementary Figure 1). Given that alterations in cell–cell adhesion are a hallmark of EMT, we next examined EMT-related markers. Western blot analysis demonstrated that *HMGA2* knockdown markedly decreased the expression of mesenchymal markers N-cadherin, Vimentin, and Slug, while concomitantly increasing the epithelial marker E-cadherin in YD8 and SCC25 cells. Together, these findings provide converging evidence that *HMGA2* promotes EMT by regulating cell adhesion programs, thereby facilitating the invasive phenotype of OSCC cells (Figure 3I, J). Since EGFR signaling has been widely implicated as an upstream regulator of EMT and is frequently activated in OSCC, we next integrated TCGA transcriptomic data with in vitro validation to examine the role of *HMGA2* in EGFR pathway regulation. Notably, network analysis of *HMGA2*-associated DEGs from TCGA revealed that multiple enriched pathways converged on EGFR as a central hub, suggesting that *HMGA2* may promote oncogenic signaling through EGFR activation. Consistently, Western blot analysis demonstrated that *HMGA2* knockdown markedly reduced EGFR expression and phosphorylation, accompanied by decreased activation of its downstream effectors, including ERK, AKT, and STAT3. Importantly, when exogenous EGF was added to *HMGA2*-silenced cells, phosphorylation of EGFR and the activation of downstream ERK and AKT pathways were restored, indicating that the loss of signaling upon *HMGA2* depletion could be rescued by EGFR stimulation (Figure 3K, L). Together, these findings provide strong evidence that *HMGA2* promotes OSCC progression by sustaining EGFR-centered oncogenic signaling cascades, thereby driving proliferation, survival, and migration.

Discussion

In this study, we investigated the role of *HMGA2* in oral quamous cell carcinoma (OSCC) by integrating TCGA with in-vitro functional assays. We demonstrated that *HMGA2* is associated with tumor progression by activating EGFR signaling, thereby facilitating epithelial-mesenchymal transition (EMT), migration, and invasion. Notably, *HMGA2* silencing reduced phosphorylation of EGFR effectors (ERK, AKT, and STAT3), and exogenous EGF partially rescued these signals, positioning

HMGA2 as an upstream facilitator of EGFR pathway activation rather than a passive downstream readout. Given that EGFR is frequently dysregulated in OSCC predominantly via overexpression or amplification [13] and that resistance to EGFR-targeted therapies remains a critical clinical hurdle, our findings suggest that *HMGA2* may function as an upstream regulator and a tractable therapeutic candidate to mitigate EGFR inhibitor resistance. We note that our study does not include *in-vivo* validation or EGFR inhibitor combination testing, which limits the current translational interpretation. To our knowledge, a direct functional link between *HMGA2* and EGFR pathway activation has not been delineated in OSCC; our data nominate *HMGA2*-high tumors as candidates for biomarker-guided EGFR-targeted combinations.

Late-stage OSCC frequently exhibits resistance to radiotherapy, chemotherapy, and targeted agents [14]. Therefore, early detection and intervention are critical for improving patient outcomes [15]. In this context, increasing attention has been directed toward identifying biomarkers that are highly expressed even in early-stage tumors [16], along with elucidating their underlying mechanisms. Moreover, strategies that incorporate combination therapies particularly those targeting well-characterized oncogenic pathways are being actively explored as promising approaches to overcome treatment resistance in refractory OSCC [17]. An important future direction will be to evaluate whether dual inhibition of *HMGA2* and EGFR yields synergistic therapeutic benefit, particularly in *HMGA2*-high OSCC models. Future studies using xenograft models and pharmacologic testing are planned to evaluate the therapeutic potential of *HMGA2*-targeted strategies.

HMGA2 is a non-histone architectural protein that influences gene transcription by interacting with AT-rich regions within the minor groove of B-form DNA, thereby inducing changes in chromatin organization [11, 10]. In the context of cancer, *HMGA2* is known to influence tumor progression by regulating DNA damage response, cell cycle control, and apoptosis [10]. Recent studies have also implicated *HMGA2* in ferroptosis through the modulation of GPX4 expression [18], suggesting its broader role in cell death regulation. Given *HMGA2*'s architectural role, one plausible mechanism is that *HMGA2* may modulate chromatin accessibility at the EGFR locus or at EGFR-ligand genes (e.g., AREG), potentially sensitizing cells to ligand-driven activation.

Although EGFR signaling is well established as a key oncogenic pathway in OSCC [13, 19, 20], the upstream mechanisms that modulate EGFR activation remain incompletely understood. Our data demonstrate that *HMGA2* functions as an upstream facilitator of EGFR pathway activation, as evidenced by reduced *ERK, AKT,* and *STAT3* phosphorylation following *HMGA2* silencing and partial rescue by exogenous EGF. Mechanistically, three non-exclusive models merit testing: (i) transcriptional upregulation of EGFR or its ligands; (ii) receptor trafficking effects on EGFR internalization/recycling; and (iii) feed-forward crosstalk via STAT3/ERK that stabilizes *EGFR* pathway output. Notably, this inhibition was rescued upon treatment with exogenous EGF, suggesting that *HMGA2* may modulate upstream components of the EGFR pathway. However, the precise mechanisms by which *HMGA2* influences EGFR signaling remain unclear. Consistent with our findings, *HMGA2* has been linked to activation of STAT3/ERK/AKT across tumor types, including colorectal, prostate cancer and AML, supporting a model in which *HMGA2* amplifies RTK-proximal signaling and EMT programs across contexts [13, 21, 22]. Taken together, these findings from various tumor contexts support the possibility that *HMGA2* influences EGFR signaling, potentially through upstream modulation and, although not directly tested, via transcriptional regulation of downstream effectors. Although our study did not determine whether *HMGA2* binds directly to the EGFR gene or engages in protein–protein interactions affecting the pathway, the observed signaling changes upon *HMGA2* knockdown raise the possibility of a direct regulatory mechanism that warrants further investigation.

Other studies have shown that non-coding RNAs such as miR-150, miR-142-3p, and LINC01121 regulate *HMGA2* expression, thereby influencing EMT, metastasis, and drug resistance in cancer [23]. Notably in OSCC, TCGA miRNA-seq and mRNA-seq allow correlation analyses between *HMGA2* and candidate ncRNAs or EGFR-axis genes, which could nominate clinically translatable ncRNA–*HMGA2* intervention points. STAT3 is an important immunomodulatory hub [24], *HMGA2*-driven EGFR–STAT3 activation may interface with an immunosuppressive TME; deconvolution of immune infiltrates versus *HMGA2* levels could clarify this axis. In cases where direct inhibition of *HMGA2* in EGFR-resistant patients is technically challenging or associated with significant adverse effects, targeting these upstream regulators of *HMGA2* may represent an alternative strategy to overcome EGFR resistance in OSCC. Such non-coding RNA-targeted approaches could be combined with therapeutic platforms such as siRNAs or miRNA mimics/inhibitors, offering considerable clinical potential in combination therapy settings [25-27].

Collectively, our findings suggest that *HMGA2* may function as an upstream regulator associated with EGFR signaling in OSCC, contributing to EMT, migration, and activation of oncogenic pathways. By integrating multi-omics analyses with functional assays, our study provides correlative and functional evidence supporting *HMGA2* as a prognostic biomarker and a tractable therapeutic candidate. This study is limited by the absence of (i) direct *HMGA2*–DNA interaction assays at EGFR/ligand loci, (ii) receptor trafficking measurements, and (iii) in-vivo validation and pharmacologic combination testing. Future work should integrate copy-number and methylation profiling of the *HMGA2* locus (12q14–15) with ChIP-qPCR/ChIP-seq at EGFR/AREG enhancers, quantify ligand transcripts and secreted proteins, and test EGFR internalization/recycling dynamics. Clinically, *HMGA2* expression could serve as a stratification marker to identify patients for *HMGA2*–EGFR vertical combinations and to prioritize trials in *HMGA2*-high, EGFR-dysregulated OSCC. Future studies should focus on validating these results in larger patient cohorts, elucidating the precise molecular interactions between *HMGA2* and EGFR pathway components, and exploring combinatorial treatment strategies that target *HMGA2* directly or through its upstream non-coding RNA regulators to overcome EGFR inhibitor resistance.

Author Contribution Statement

Jun Wu conceived and designed the study, supervised the project, and revised the manuscript. YaTian Liu performed the experiments, analyzed the data, and drafted the manuscript. Xiao Jiao assisted with data analysis and experimental validation. All authors reviewed and approved the final version of the manuscript.

Acknowledgements

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki.

The transcriptomic and clinical data were obtained from The Cancer Genome Atlas (TCGA), a publicly available database with all patient information de-identified; therefore, no additional ethical approval or informed consent was required.

All in vitro experiments were performed using established cell lines and did not involve human participants or animals.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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