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EGFL6 promotes endometrial cancer cell migration and proliferation

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HIGHLIGHTS

- EGFL6 is upregulated in endometrial cancer, primarily in copy-number high tumors.
- EGFL6 upregulation in endometrial cancer portends poor prognosis.
- EGFL6 increases endometrial cancer cell proliferation and migration.
- Loss of EGFL6 significantly restricts endometrial cancer growth.
- EGFL6 represents a potential therapeutic target for poor prognosis endometrial cancer.

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ABSTRACT

Objective. EGFL6, a growth factor produced by adipocytes, is upregulated in and implicated in the tumorigenesis of multiple tumor types. Given the strong link between obesity and endometrial cancer, we sought to determine the impact of EGFL6 on endometrial cancer.

Methods. EGFL6 expression in endometrial cancer and correlation with patient outcomes was evaluated in the human protein atlas and TCGA. EGFL6 treatment, expression upregulation, and shRNA knockdown were used to evaluate the impact of EGFL6 on the proliferation and migration of 3 endometrial cancer cell lines in vitro. Similarly, the impact of EGFL6 expression and knockdown on tumor growth was evaluated. Western blotting was used to evaluate the impact of EGFL6 on MAPK phosphorylation.

Results. EGFL6 is upregulated in endometrial cancer, primarily in cony-number high tumors. High tumor endometrial cancer expression of EGFL6 predicts poor patient prognosis. We find that EGFL6 acts to activate the MAPK pathway increasing cellular proliferation and migration. In xenograft models, EGFL6 overexpression increases endometrial cancer tumor growth while EGFL6 knockdown decreases endometrial cancer tumor growth.

Conclusions. EGFL6 is a marker of poor prognosis endometrial cancers, driving cancer cell proliferation and growth. As such EGFL6 represents a potential therapeutic target in endometrial cancer.

1. Introduction

Endometrial cancer is the most common gynecologic malignancy in the United States with an estimated 66,200 new diagnoses and 13,030 deaths attributable to uterine cancer in 2023 [1]. Risk factors for development of endometrial cancer include prolonged unopposed estrogen exposure, obesity, and increasing age [2]. Over the past 15 years, both the incidence and mortality rates of uterine cancer have steadily increased both in the US and world-wide [3].

Classically, endometrial cancers have been divided into 2 types. Type 1 cancers with endometrioid histology are more common and most associated with increased estrogen exposure and obesity. Type 2 cancer, including high grade serous and clear cell histologies, are generally much more aggressive but not thought to be estrogen linked. More recently, advancements in the understanding of pathogenic and molecular features of endometrial cancer, largely propelled by the publication of the Cancer Genome Atlas, has transitioned focus from the two-tier system to a molecular classification system based on copy number and POLE, MMR, and p53 mutations [4]. Rates of endometrial cancers

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are rising, particular among non-Hispanic blacks [5]. The increasing incidence of endometrial cancer is strongly correlated with increasing obesity [3]. Indeed, of all malignancies, endometrial cancer is the cancer most strongly linked with obesity [6]. Interestingly, while increased obesity is thought to increase estrogen exposure, both type 1 (estrogen linked) and type 2 (non-estrogen linked) tumors have been correlated with obesity [5]. Indeed, other signaling pathways have been clearly linked with obesity and endometrial cancer with additional understanding of the molecular pathways driving obesity and the development of endometrial cancer being necessary [4].

Patients diagnosed with early-stage endometrial cancer are often cured with surgery alone and have excellent prognoses. However, patients with advanced or recurrent disease carry poor prognoses [5]. While recent studies show a benefit for immune checkpoint inhibitors in endometrial cancer [6–8], clearly new therapeutic approaches are needed for advanced stage endometrial cancer. One potential therapeutic target is the epidermal growth factor-like domain 6 (EGFL6) protein. EGFL6, a member of the EGF-like repeat superfamily, is a secreted protein that plays an important role in development [9–11]. EGFL6 is also expressed in adipose tissue, playing a role in adipose differentiation and thus could be a potential link between obesity and endometrial cancer [12]. Indeed, increasing evidence indicates EGFL6 plays an important role in cancer initiation and growth. EGFL6 is commonly upregulated in cancer, thereby increasing angiogenesis, tumor growth, and metastasis [13–16].

Here we sought to determine if EGFL6 could play a role in endometrial cancer initiation and growth. We find increased EGFL6 expression in endometrial cancer is highly correlated with poor prognosis, with the highest EGFL6 expression in type-2 endometrial cancers. Furthermore, we find that EGFL6 activates signaling of the MAPK pathway, increasing cancer cell proliferation and migration in vitro and increasing tumor growth in vivo. Supporting EGFL6 as a therapeutic target, EGFL6 knockdown in EGFL6 expressing cell lines, significantly restricted tumor growth. These findings offer evidence that EGFL6 represents a potential therapeutic target in endometrial cancer.

2. Materials and methods

Bioinformatics analysis: Overall survival data according to EGFL6 expression were obtained from Human Protein Atlas (https://www.proteinatlas.org/). For high vs. low expression in the human protein human protein atlas analysis, the expression which best delineates two groups of expression is automatically selected by the website. The cutoff chosen was 4.95 FKPM – essentially defining very low/no expression vs. upregulated expression. Data for EGFL6 expression analysis in different endometrial cancer histologies were obtained from Oncomine (https://www.oncomine.org). EGFL6 expression in specific endometrial cancer cell lines was obtained from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle). For TCGA data analysis.

cBioPortal (https://www.cbioportal.org) was used for EGFL6 expression using Uterine Corpus Endometrial Carcinoma (UCEC) Datasets. Survival and clinical attributes were compared between EGFL6-altered (EGFL6: GAIN EXP > 1.5, EXP Z score > 2) and the remaining (unaltered) cases using RNAseq root mean square error (RMSE) and putative copy-number alterations data.

Cell culture: Ishikawa, MFE-280 and HEC-1-A cell lines were obtained from the Reuda lab (Massachusetts General Hospital, Boston MA). The Ishikawa cell line was established from a well differentiated endometrioid adenocarcinoma from a 39-year-old patient and is associated with PTEN mutations [17,18]. The MFE-280 cell line was established from a poorly differentiated endometrioid adenocarcinoma recurrence in a 78-year-old patient and is associated with TP53 mutations [17,19]. The HEC-1-A cell line was established from an endometrioid adenocarcinoma from a 71-year-old and is associated with MSH6/PMS2 mutations [17]. Identity of the cultured cell lines was confirmed by STR testing via ATTC. MFE-280 and Ishikawa cell lines were maintained in MEM, and HEC-1-A cells were maintained in McCoy's 5 A media. The media was supplemented with 10% FBS and 1% Penicillin/Streptomycin, and cells were maintained and grown at 37 °C and 5% CO_2. When defining EGFL6 RNA expression for the endometrial cancer cell lines in this study, we compared their RNA expression to that of EGFL6-deleted SKOV3 ovarian cancer cell lines and then among the cell lines themselves.

Cell proliferation assays: Cell lines were plated in triplicate in 96-well plates in growth factor-free spheroid media. Cells were plated at 5000–10,000 cells/ml and treated with 200 ng/ml recombinant EGFL6 daily for three days. Total cell number was assessed 4 days after cell seeding.

Tumor spheroid culture: Cell lines were plated in triplicate in ultra-low attachment plates in growth factor-free spheroid media as previously described [13]. Cells were plated at 5000–10,000 cells/ml. Sphere formation and total sphere count were assessed 7 days after cell seeding.

Quantitative real-time PCR (qRT-PCR): qRT-PCR was performed to compare the expression of EGFL6 in the endometrial cancer cells lines used in this study compared to EGFL6 non-expressing SKOV3 cell line (negative control). Total RNA was extracted using RNeasy Mini Kit (Qiagen), and cDNA was synthesized using the Superscript III First-Strand System (Invitrogen). qRT-PCR reactions were performed in triplicate with SYBR Green (AB Biosystems) with each experiment repeated three times. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method with YWHAZ as an internal control. See Supplemental Table 1 for MIQE information [20].

Western blot analysis: Cells were washed twice with PBS and then lysed using radioimmunoprecipitation assay (RIPA) buffer and supplemented with halt protease inhibitor cocktail (ThermoScientific). Total protein concentrations were determined using BCA Protein Assay (ThermoScientific). Samples with equal protein amounts were then separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes, and blotted using antibodies. The primary antibodies were anti-phosphorylated MAPK p44/42 (1:1000, Cell Signaling Technology Inc., Massachusetts), anti-total MAPK p44/42 (1:1000, Cell Signaling Technology Inc., Massachusetts) and anti-GAPDH (1:1000, Proteintech). Secondary antibodies were HRP-conjugated anti-rabbit (1:5000, Sigma-Aldrich) and HRP-conjugated anti-mouse (1:5000, Sigma-Aldrich). The loading control was GAPDH, and the negative controls were untreated cell lysates.

EGFL6 shRNA knockdown: Ishikawa and MFE-280 cells were transduced with shRNA targeting EGFL6 or control as previously described [13] and selected with puromycin. qRT-PCR was performed (as above) to confirm EGFL6 knockdown.

EGFL6 overexpression: HEC-1-A cells were transduced with EGFL6 or control through lentiviral infection followed by FACS sorting of GFP-positive cells. qRT-PCR was performed to confirm EGFL6 overexpression.

Wound healing assay: Cells were plated in 60 mm culture dishes and incubated for 24 h until confluent. Using a 1000ul pipette tip, the cell layer was disrupted in a linear fashion. Images were obtained at 0 and 20 h post scratch. Percent wound closure was calculated as the total wound area at 20 h divided by the total wound area at 0 h × 100.

Cell migration assays: Cells were seeded on the top chambers of a transwell plate at a density of 7×10^5 cells/well (8 μm pores, Corning Inc., Corning NY) in media with 2% FBS, and the bottom chamber was filled with media 2% FBS and 400 ng/ml EGFL6. Cells in the top chamber were removed after 24 h, fixed, stained with crystal violet and counted.

Tumor sphere assay: HEC-1-A cells 1000 were plated in low-attachment plates with serum free MEBM supplemented with 5 μg/ml insulin, and 10 ng/ml β-FGF. After 5 days CellTiter-Glo (Promega) was used for Tumor sphere growth and viability analysis.

In vivo studies: Animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Tumors were generated as previously described [21]. Briefly, one

EGFL6-or empty-vector transduced cells were injected into bilateral mouse axilla at 1×10^6 cells per injection suspended at a 1:1 ratio of PBS and matrigel. Tumor volume was monitored twice weekly, and mice were euthanized when one or more tumors reached approximately 1000 mm^3. Similarly, EGFL6-shRNA or scrambled-shRNA control cells were injected into bilateral mouse axilla at 1×10^6 cells per injection as above. At the time of euthanasia, mice were evaluated for gross metastases.

Statistical analysis: The data are expressed as means ± standard deviation. Comparisons between groups were performed using the Student's *t*-test (two-tail, $p < 0.05$) using GraphPad Prism 6 (San Diego, CA) software.

3. Results

EGFL6 expression is upregulated in endometrial cancer and associated with poor prognosis.

Datasets available from the Oncomine Cancer Microarray database (https://www.oncomine.org) were used to determine EGFL6 expression in endometrial cancers. Cancer outlier profile analysis (COPA) indicated EGFL6 was expressed in the 75th percentile of genes across four endometrial cancer datasets (Fig. 1A). Analysis by histologic subtypes indicated that while both Type-I and Type-II endometrial cancers express EGFL6, Type-II cancers express significantly more EGFL6 compared to Type I endometrial cancers (Fig. 1B). The available dataset did not include additional information regarding molecular classification for further analysis. We next used the Human Protein Atlas (www. https://www.proteinatlas.org) to analyze overall survival by level of EGFL6 protein expression in endometrial cancers. Consistent with reports in other tumor types [13,14], high EGFL6-expressing tumors were associated with worse overall survival compared to low EGFL6-expressing tumors ($p = 0.0038$) (Fig. 1C). We similarly analyzed the EGFL6 expression in human endometrial cancer subsets. Increased EGFL6 expression was associated with both poor progression free and overall survival (Fig. 1D). When EGFL6 expression was stratified by molecular subtypes, increase EGFL6 was most highly associated with the poor prognosis copy number high tumors and least associated with the good prognosis POLE subset (Fig. 1E).

3.1. EGFL6 promotes endometrial cancer cell proliferation

We next screened the Cancer Cell Line Encyclopedia (CCLE) (https://portals.broadinstitute.org/ccle) to identify EGFL6 expressing endometrial cancer cell lines (Supplemental Fig. 1A). We used qRT-PCR to confirm mRNA expression in three endometrial cancer cell lines: HEC-1-A, Ishikawa and MFE-280. Consistent with the CCLE data, we found that HEC-1-A cells have extremely low EGFL6 mRNA expression, Ishikawa cells have intermediate expression, and MFE-280 cells have high expression when compared to EGFL6-deleted SKOV3 ovarian cancer cells (Fig. 1F).

EGFL6 is reported to promote proliferation of ovarian, breast and other cancer cell types [13–15]. To evaluate the effect of EGFL6 on endometrial cancer cell proliferation, we performed cell proliferation assays for each endometrial cancer cell line in the presence/absence of recombinant human EGFL6. The EGFL6 low/non expressing HEC-1-A and Ishikawa cells demonstrated a statistically significant increase in total cell number, while MFE-280 cells, which likely make an overwhelming level of EGFL6, demonstrated no difference in total cell number after recombinant EGFL6 treatment (Fig. 2A i-iii).

As EGFL6 has been linked with cancer stemness, we next performed tumor spheroid assays, a common stemness assay, for each endometrial cancer cell line. With recombinant EGFL6 treatment, HEC-1-A and Ishikawa cells demonstrated a significant increase in total tumor spheroid number, and MFE-280 cells demonstrated a trend toward an increase in total tumor spheroid number (Fig. 2B).

To validate these results, we next overexpressed EGFL6 in HEC-1-A cells (HEC-1-A^{EGFL6}) using a lenti-vector transduction system. qRT-PCR was performed to confirm EGFL6 overexpression (Fig. 2C i). Cell proliferation assays were then repeated using the HEC-1-A^{EGFL6} cells compared to HEC-1-A control. Compared to vector controls, HEC-1-A^{EGFL6} cells demonstrated significantly increased total cell number in both 2D cell culture (Fig. 2C ii) and in 3D tumor spheroid assays (Fig. 2C iii-iv), with the greatest impact observed in the spheroid assay.

In order to evaluate the effect of EGFL6 loss on proliferation of endometrial cancer cell lines, we next knocked down EGFL6 in Ishikawa and MFE-280 using previously validated EGFL6 shRNA [14]. qRT-PCR confirmed EGFL6 knockdown with shRNA-2 being the most effective (Fig. 2D i-ii). Tumor spheroid assays were then performed using the EGFL6 knockdown cell lines. EGFL6 knockdown in Ishikawa cells demonstrated significantly ($p < 0.007$) reduced total tumor spheroid number compared to controls. EGFL6 knockdown resulted in a decrease in total tumor spheroid number ($p \leq 0.07$) compared to controls (Fig. 2E i-ii).

3.2. EGFL6 promotes endometrial cancer cell migration

EGFL6 is reported to promote cancer cell metastasis by increase cancer cell migration [15]. To determine the effect of EGFL6 on endometrial cancer cell migration, transwell migration assays were performed with the addition of recombinant EGFL6 to the bottom chamber of the transwell. Addition of recombinant EGFL6 resulted in a 2–4 fold increase in cell migration of all three endometrial cancer cell lines tested (Fig. 3A i-iii).

The effect of EGFL6 on endometrial cancer cell migration was then confirmed using wound healing assays. We first used the low EGFL6 expressing HEC1 A cells. Treatment of HEC-1-A cells with recombinant EGFL6 resulted in significantly increased cell migration compared to vehicle treated controls (Fig. 3B). Similarly, using the Ishikawa and MFE-280 without and with EGFL6 shRNA knockdown, we demonstrated significantly decreased wound closure with EGFL6 knockdown (Fig. 3C i-ii).

3.3. EGFL6 signaling activates the p-ERK pathway and promotes endometrial tumor growth

To examine EGFL6 cellular signaling pathways, HEC-1 A, Ishikawa and MFE-280 parental cell lines were treated with recombinant EGFL6 and subsequently collected at varying time points for Western blot analysis (Fig. 4A i-iii). We found that, similar to that seen in ovarian cancer cells, EGFL6 exposure results in an increase in p-MAPK at 7.5 min compared to no treatment controls for all three cell lines. We then treated HEC-1-A tumor spheres with two MAPK inibitors, Mirdametinib and Trametinib, in the presence of absence of EGFL6 and examined tumor spheroid growth. We found that MAPK inhibition attenuated EGFL6 induced tumor spheroid growth (Fig. 4B), These results demonstrate that EGFL6 signals at least in part through the MAPK pathway.

We next evaluated the in vivo effects of EGFL6 expression on tumor growth in vivo. We evaluated tumor growth of HEC-1-A^{EGFL6} overexpressing clones compared to HEC-1-A clones. HEC-1-A^{EGFL6} clones demonstrated significantly increased tumor growth compared to controls (Fig. 4B). Conversely, we found that EGFL6 knockdown significantly reduced tumor growth of both MFE-280 and Ishikawa cells; EGFL6 knockdown in EGFL6 high expressing MFE-280 cells resulted in a ~ 8-fold reduction in tumor volume and weight (Fig. 4C i-ii), while knockdown in the EGFL6 intermediate expressing level Ishikawa cells resulted in a 2–3 fold reduction in tumor growth (Fig. 4D i-ii).

4. Discussion

In this study, we demonstrate that EGFL6 increases endometrial cancer cell proliferation and migration to ultimately promote tumor growth. Consistent with this knockdown of EGFL6 decreases endometrial cancer cell proliferation, migration, and tumor growth. The results

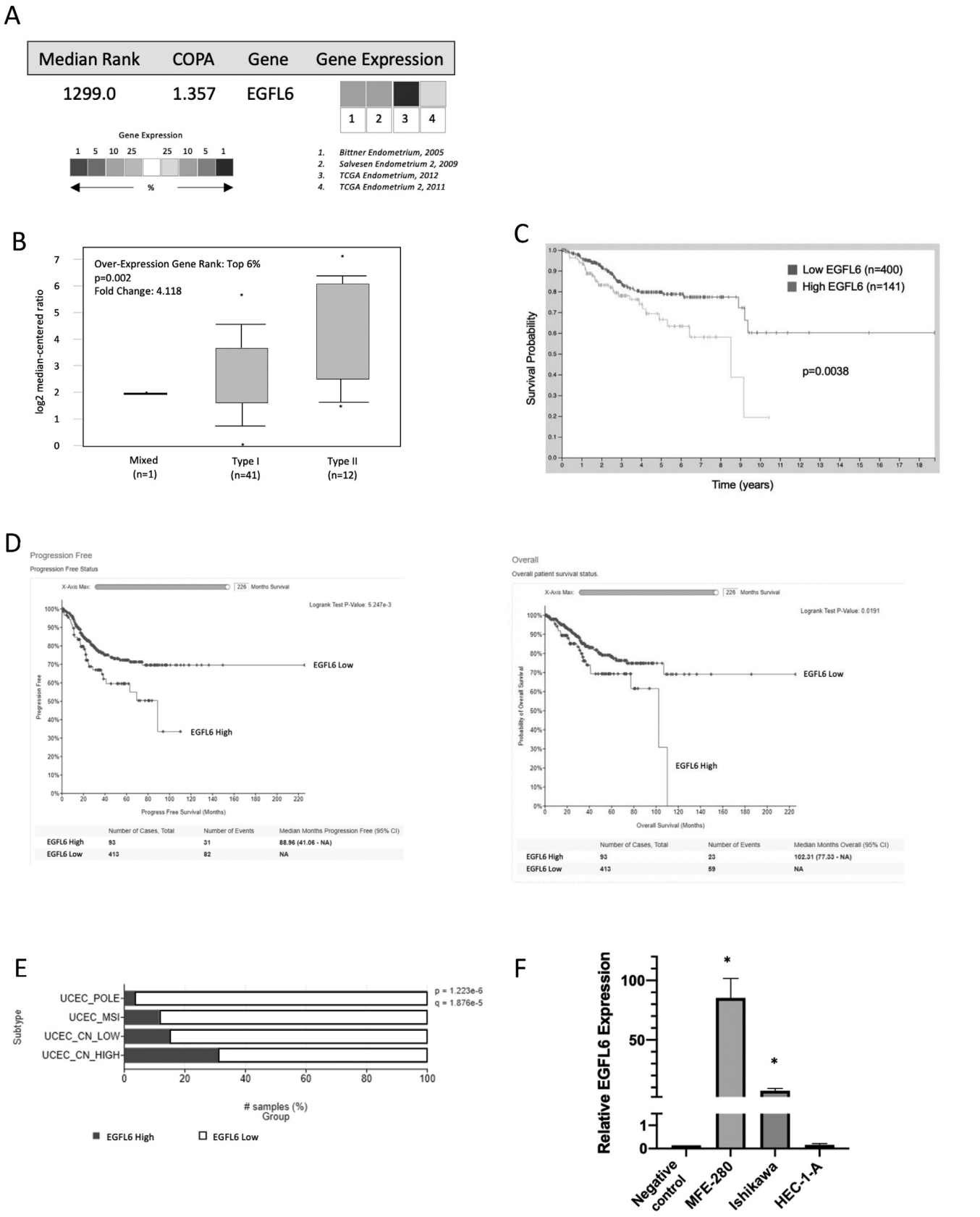

Fig. 1. EGFL6 expression in endometrial cancer. A. Cancer outlier profile analysis (COPA) analysis of EGFL6 overexpression across four endometrial cancer datasets (Oncomine). B. Expression of EGFL6 across endometrial cancer histologies in the TCGA dataset (Oncomine). C. Overall survival analysis for patients with EGFL6 high and low expressing endometrial cancers from the Human Protein Atlas. D. Progression free and overall survival of endometrial for patients with EGFL6 high and low expressing endometrial cancers using the TCGA dataset. E. EGFL6 expression stratified by molecular subtypes showing EGFL6 expression is greatest in copy number (CN) high tumors. F. qRT-PCR analysis of EGFL6 expression in endometrial cancer cell lines as compared to EGFL6 non-expressing SKOV3 cell line (negative control).

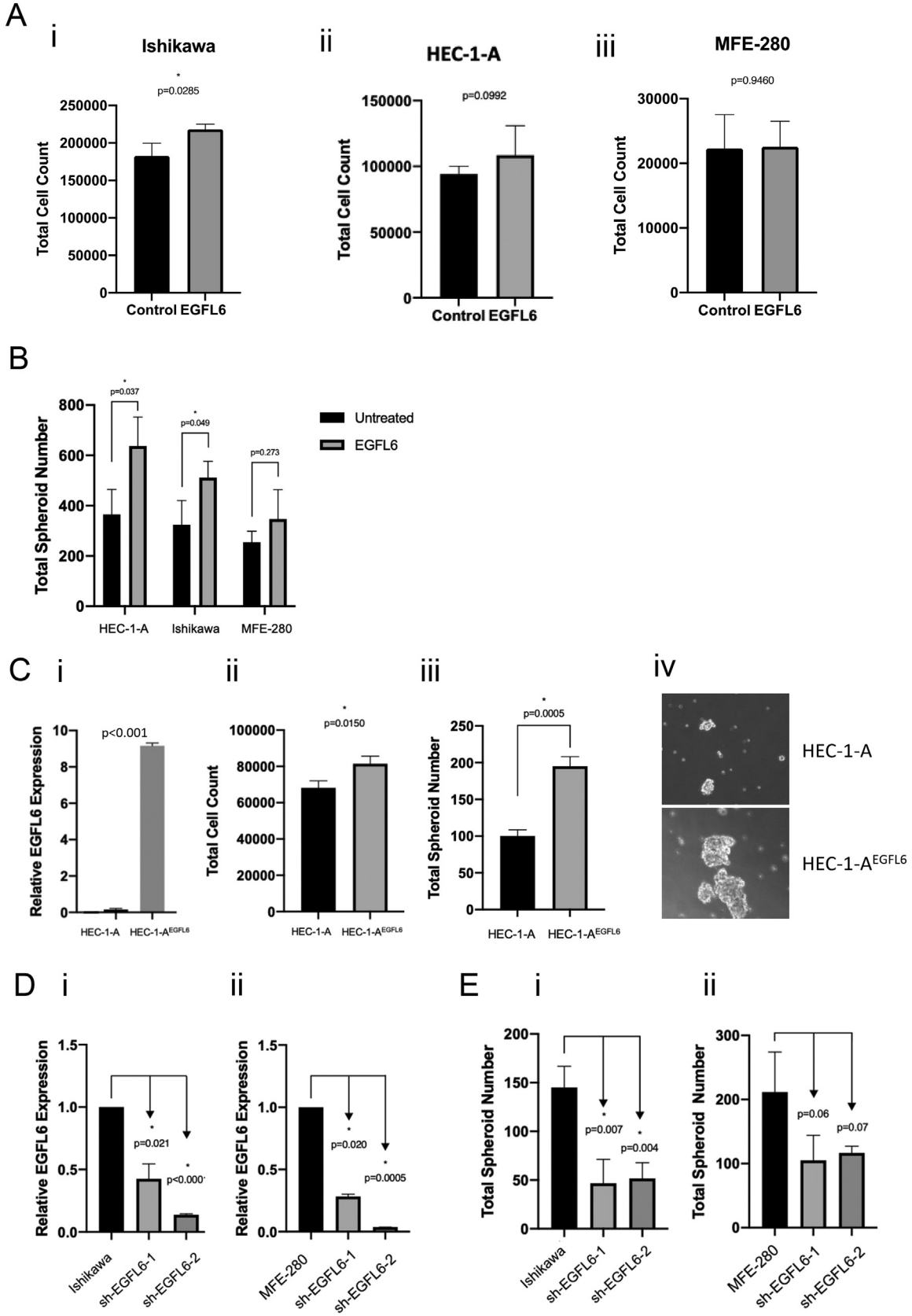

Fig. 2. EGFL6 increase endometrial cancer cell proliferation in both 2D and tumor spheres. A. Total cell numbers of (i) Ishikawa, (ii) HEC-1-A, and (iii) MFE-280 cells in the absence and presence of EGFL6. B. Total spheroid number in the indicated control and recombinant EGFL6 treated endometrial cancer cell lines. C (i) qRT-PCR confirmation of EGFL6 overexpression in HEC-1-A^{EGFL6} versus HEC-1-A control, (ii) total cell count in HEC-1-A versus HEC-1-A^{EGFL6} cells, and (iii) tumor spheroid growth in HEC-1-A versus HEC-1-A^{EGFL6} (iii). D i-ii. qRT-PCR analysis of EGFL6 mRNA expression in control Ishikawa and MFE280 cells with two independent EGFL6 shRNA. E. Tumor spheroid growth in (i) Ishikawa and (ii) MFE-280 without and with EGFL6 shRNA knockdown. Three biologic and technical replicates were performed for all experiments. Error bars are standard deviations.

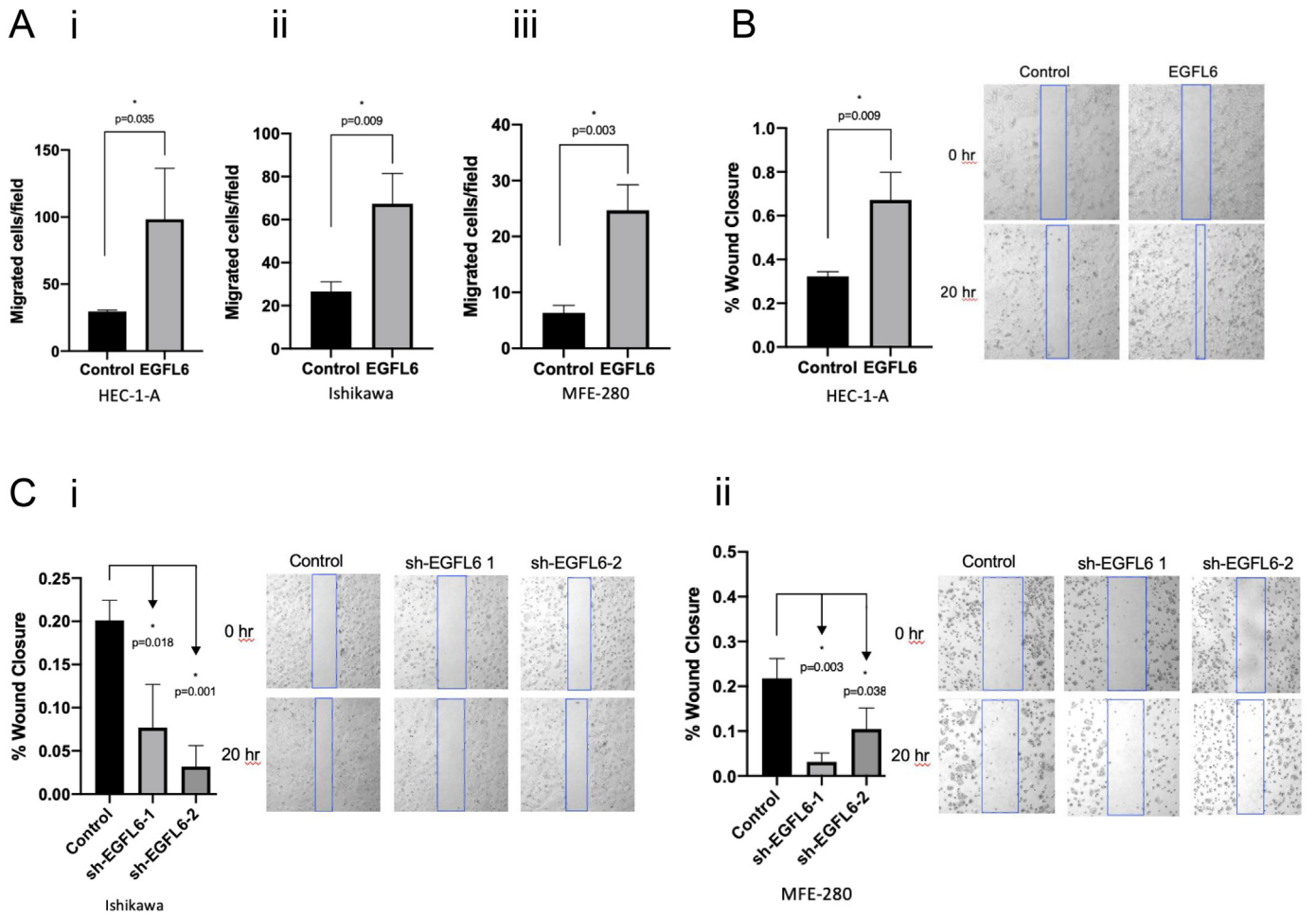

Fig. 3. EGFL6 promotes endometrial cancer cell migration. A. Summary of recombinant EGFL6 stimulated cell migration using transwell assays for (i) HEC-1-A, (ii) Ishikawa, (iii) MFE-280 cell lines. B. Average wound closure and representative images for HEC-1-A control and HEC-1-A-EGFL6 overexpressing cell lines. C. Average wound closure and representative images for (i) Ishikawa (ii) MFE280 cells without and with EGFL6 shRNA knockdown. Three biologic and technical replicates were performed for all experiments. Error bars are standard deviations.

of this study provide evidence that EGFL6 is a critical growth factor for endometrial cancer cells and may represent a viable therapeutic target in endometrial cancer.

To date, the role of EGFL6 in tumorigenesis has been primarily elucidated in other cancer types. High EGFL6 expression is associated with worse prognosis when compared to low EGFL6 expression in ovarian, breast and colon cancer [13–15,22]. In addition, EGFL6 serum levels correlated with advanced stage and distant metastasis in nasopharyngeal cancer [23]. The impact of EGFL6 on tumor growth are many fold. EGFL6 is expressed in tumor vasculature and is an important angiogenic factor [13,16,24–26]. In addition to a role for EGFL6 in angiogenesis, we and others reported that EGFL6 is also expressed in ovarian cancer cells and increases ovarian cancer cell proliferation and tumor growth by regulating the ovarian cancer stem-like asymmetric division [13,27]. An et al. demonstrated that EGFL6 induces an epithelial-mesenchymal transition of breast cancer and maintains the population of breast cancer-like stem cells [15], and Huo et al. confirmed similar findings in gastric cancer cells [28].

EGFL6 signaling has been found to signal through several different pathways. In colorectal cancer, EGFL6 was found to increase cancer cell proliferation through activation of ß-catenin via the WNT pathway [14]. In breast cancer, EGFL6 signaling involves activation of Akt and MAPK [15], and in gastric cancer EGFL6 promotes cancer cell invasion and metastasis through activation of the PI3K, notch and MAPK signaling pathways [28]. The present study confirms EGFL6 signaling through the MAPK pathway in endometrial cancer cells. This is of particular importance given that obese patients with endometrial cancer often have insulin resistance and hyperinsulinemia which have been found to potentiate the effects of IGF-1 leading to hyperactivation of intracellular pathways including MAPK [29].

Several studies have demonstrated that anti-EGFL6 therapy or EGFL6 blockade results in decreased cancer cell proliferation and tumor growth in multiple tumor types [13,15]. Noh et al. demonstrated that silencing of EGFL6 resulted in a reduction in ovarian cancer tumor growth but did not affect wound healing and concluded that anti-EGFL6 therapy represents a promising therapeutic target for ovarian cancer with the potential for mitigation of specific toxicities associated with antiangiogenic therapies currently available [16]. Similarly we found that both murine and human anti-EGFL6 antibodies can restrict cancer growth [13,30]. The present study provides further evidence that anti-EGFL6 therapy may represent a viable therapeutic target.

In conclusion, we demonstrated that EGFL6 promotes endometrial cancer cell proliferation and migration as well as tumor growth through activation of the MAPK signaling pathway. This suggests that EGFL6 is an important tumor growth factor involved in endometrial cancer tumor growth. This study provides evidence that EGFL6 represents a potential therapeutic target in endometrial cancer and that further investigation of anti-EGFL6 therapy is warranted.

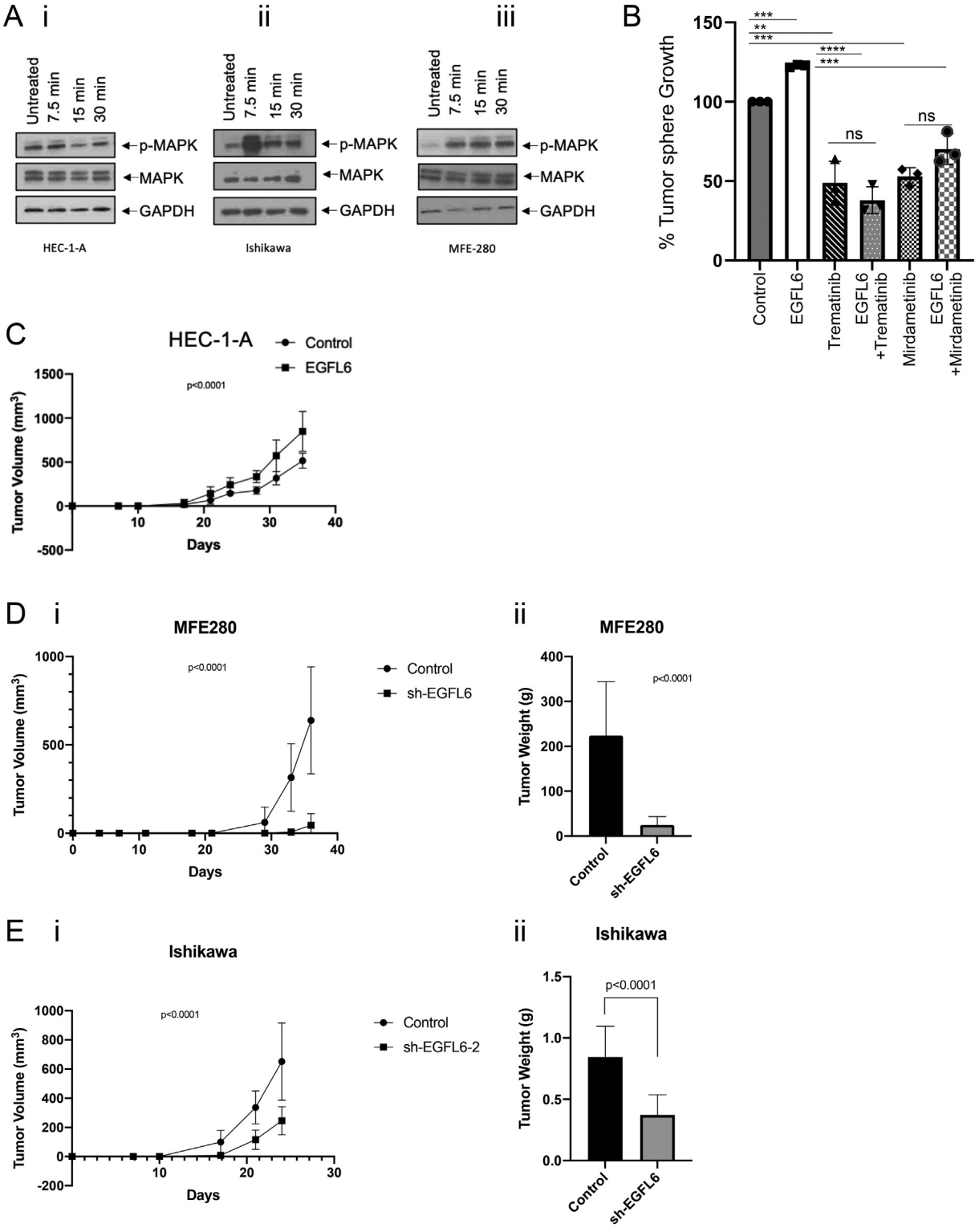

Fig. 4. EGFL6 promotes tumor growth in vivo. A. Western blot for p-ERK and total ERK in (i) HEC-1-A, (ii) Ishikawa, and (iii) MFE280 cell lines after treatment with recombinant EGFL6 for the indicated durations. B. MAPK Inhibitors attenuated EGFL6 induced tumor sphere growth. HEC1A tumor spheres were treated with two MAPK inhibitors Mirdametinib (100 nM) or Trametinib (100 nM) in the presence or absence of EGFL6 (400 ng/ml) for 5 days. Experiments were repeated three times in triplicates. Student t –test was used for statistic analysis. *:$p < 0.05$; **:$p < 0.01$; ***:$p < 0.001$; ****:$p < 0.0001$. C. Tumor growth curves of HEC-1-A and HEC-1-A^{EGFL6} cells. D. (i) Tumor growth curves and (ii) tumor weights of MFE-280 control and MFE280-sh-EGFL6 cells. E. (i) Tumor growth curves and (ii) tumor weights of Ishikawa control and Ishikawa-sh-EGFL6 cells. Three biologic replicates were performed for each of these experiments.

Author contributions

AG, SB, SC, NG, DY all contributed to experimental design, data collection and analysis, and reviewed the manuscript. AG and RB were involved in primary manuscript generation. RB provided conceptualization, guided experimental design, and obtained funding for the work.

CRediT authorship contribution statement

Alison A. Garrett: Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **Shoumei Bai:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **Sandra Cascio:** Data curation, Formal analysis, Methodology. **Navneet Gupta:** Conceptualization, Data curation, Formal analysis, Methodology. **Dongli Yang:** Data curation, Formal analysis, Methodology. **Ronald J. Buckanovich:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

Dr. Buckanovich is co-founder of Tradewind Bioscience which is developing EGFL6 targeted therapy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2024.02.016.

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