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Inhibition of HMGA2 Leads to Reduced Cell Proliferation and Increased Apoptosis in Human Embryonal Carcinoma Cell Lines

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ABSTRACT

Background: The most prevalent solid tumors in young men are testicular germ cell tumors (TGCTs), and embryonal carcinoma is the most common subtype among non-seminomatous germ cell tumors (NSGCTs). Despite the excellent cure rates of cisplatin-based chemotherapy, resistance develops in 15%–30% of patients with metastatic cancer, which results in a poor prognosis. The overexpression of the High Mobility Group A2 (HMGA2) protein has been linked to treatment resistance and cancer aggressiveness. It is well known that this protein promotes carcinogenesis.

Objective: The purpose of this work was to investigate the functional role of HMGA2 in EC cell migration, survival, and proliferation, focusing on its role as a potential therapeutic target in cisplatin-resistant ECs.

Materials and Methods: We employed human EC cell lines EP2102 and GCT27, as well as the cisplatin-resistant (CisR) versions of these cell lines that were produced by prolonged drug treatment. siRNA transfection was used to suppress *HMGA2* expression. Growth curve and colony formation tests were used to measure cell proliferation. Apoptosis was assessed by Annexin V staining followed by flow cytometry, cell cycle distribution was analyzed by flow cytometry, and cell migration was detected by Boyden Chamber Assays.

Results: In both parental and resistant EC cell lines, *HMGA2* knockdown markedly decreased proliferation. After *HMGA2* knockdown, flow cytometric analysis revealed S phase arrest. Apoptosis was significantly elevated, especially in cells that were resistant to cisplatin. In addition, all HMGA2-depleted cell lines showed decreased migration. These impacts were true for both the GCT27 and EP2102 models.

Discussion: The data suggest that HMGA2 is necessary to preserve the EC cells' malignant characteristics. Its silencing interferes with several cancer-related functions, including motility, survival, and cell cycle progression. These results indicate HMGA2's participation in chemoresistance mechanisms and are consistent with its role in other solid cancers.

Francesco Esposito and Paolo Chieffi share last authorship.

Conclusion: Our results indicate a role of HMGA2 in the EC, because its inhibition reduces cell malignant characteristics, and may represent a viable therapeutic target to improve the prognosis of CisR TGCTs.

1 | Introduction

The most prevalent solid tumors in adolescents and young men aged 20–40 are testicular germ cell tumors (TGCTs) [1]. TGCTs are divided into two histological groups, seminoma and non-seminoma, which include yolk sac tumors, choriocarcinoma, teratoma, embryonal carcinoma (EC), and occasionally seminoma [1, 2]. Of them, EC is the most prevalent non-seminomatous germ cell tumor (NSGCT), occurring in around 77% of mixed NSGCTs [3]. Cisplatin-based chemotherapy has dramatically improved the prognosis of TGCTs, leading to cure rates exceeding 80%, even in patients with disseminated disease [4, 5]. However, despite these remarkable outcomes, 15%–30% of patients with metastatic TGCTs fail to achieve durable remission and eventually relapse with progressive, treatment-refractory disease, which is associated with poor prognosis and high mortality [6, 7]. Our understanding of the molecular mechanisms linked to TGCTs patients' resistance to cisplatin-based chemotherapy is still somewhat restricted, despite the fact that these pathways have been thoroughly investigated [8–10].

Cancer treatment relies on multiple therapeutic strategies aimed at eliminating malignant cells while limiting toxicity to normal tissues. Chemotherapy, with surgery and radiation, constitutes one of the primary modalities employed in the treatment of neoplastic illnesses. Nonetheless, it often proves ineffective as chemotherapeutic agents often cannot differentiate between normal and malignant cells, leading to the selection of resistant cell populations that multiply and grow, ultimately resulting in a suboptimal treatment response. Moreover, resistant cells exhibit increased aggressiveness and a heightened propensity for metastasis, resulting in diminished patient survival [11, 12]. In this context, identifying molecular determinants of chemoresistance is essential to improve therapeutic efficacy.

Classified as "architectural transcription factors", high mobility group AT-hooks 1 and 2 (HMGA1 and HMGA2) are essential for the remodeling of chromatin structure and the control of DNA binding transcription factors [13, 14]. In particular, *HMGA2* has been extensively implicated in cellular transformation and tumor progression and is frequently overexpressed in both spontaneous and carcinogen- or virus-induced tumors [15]. Furthermore, human and mouse cancer cells that overexpress *HMGA2* promote the development of colonies, invasion, and metastasis of cancer cells [16, 17]. On the other hand, HMGA2 depletion causes both programmed cell death and an inhibition of cancer cell growth [15, 17]. We have previously showed that *HMGA2* is strongly expressed in meiotic and post-meiotic cells and that it has a specialized role in regulating spermatogenesis, as this differentiation mechanism is significantly disrupted in mice lacking *HMGA2* [18]. In addition, we have discovered an *HMGA2* upregulation in human EC [14]. Accumulating evidence indicates that aberrant expression of HMGA proteins contributes to the development of chemoresistance [19]. While several studies have highlighted the involvement of HMGA family members in modulating drug response, the role of HMGA2 in cisplatin resistance in TGCTs remains poorly defined. One of the biggest clinical problems is cisplatin resistance, and a full comprehension of the processes that lead to TGCTs resistance may increase the success rate of TGCTs treatment. Numerous cisplatin resistance mechanisms have been discovered, most of which are connected to methylation, DNA repair systems, tumor cell cycle control, and apoptotic pathways [20, 21]. In addition to testicular tumors, other solid tumors like ovarian, breast, head and neck, and non-small cell lung cancers, where cisplatin-based chemotherapy is still the first line of treatment, may benefit from improved treatment outcomes if new therapeutic targets are found based on the molecular and genetic features of chemoresistant TGCTs [22].

In the present study we investigate the role of HMGA2 in EC and its association with cisplatin resistance. To this aim, we have used cisplatin-resistant variants of EC cell lines, namely EP2102 and GCT27, derived from established cell lines by a long-term exposure of the parental cells to the cisplatin in vitro, designated as EP2102 CisR and GCT27 CisR [23], characterized with high *HMGA2* expression associated with a cancer phenotype.

2 | Results

2.1 | TCGA Analysis Confirms Distinct HMGA2 Expression Patterns in Seminomas and ECs

We examined RNA-seq data from The Cancer Genome Atlas (TCGA) to further validate that *HMGA2* is upregulated in ECs and downregulated in seminomas. Consistent with previously published data [14], the results demonstrated a significant upregulation of *HMGA2* expression in ECs and a marked downregulation in seminomas (Figure 1). Although a variable expression trend was noted for yolk sac tumors and teratomas, the small number of available samples precluded statistically significant analysis.

2.2 | HMGA2 Arrests EC Cell Proliferation, Blocking Them in the S Phase of the Cell Cycle

We evaluated the role that HMGA2 plays in EC cellular proliferation, by silencing its expression. At first, we were able to suppress the expression of *HMGA2* in EP2102 and GCT27, as well as in their cisplatin-resistant versions, transfecting them with HMGA2 small interfering RNAs (siRNAs) (Figure 2).

Following that, we carried out a colony-forming test in order to ascertain whether or not the downregulation of *HMGA2* has an effect on the proliferation of EC cells. According to the data presented in Figure 3, the number of colonies that were produced by HMGA2-silencing cells was much lower than that of the control cells. Indeed, in EC cell lines that were sensitive to

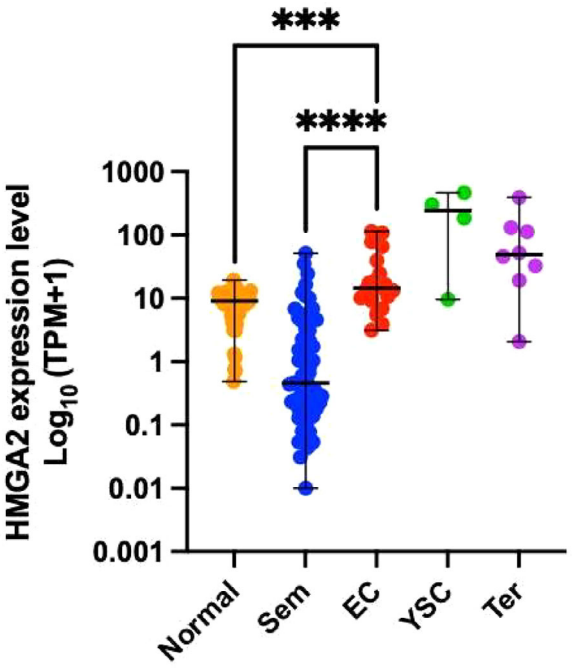

FIGURE 1 | Differential expression of *HMGA2* across testicular germ cell tumor subtypes. TCGA data showing *HMGA2* mRNA expression level in 60 normal samples, 64 seminomas (Sem), 24 embryonal carcinomas (EC), 4 yolk sac tumors (YST), and 8 teratomas (Ter).

cisplatin and those that were resistant to it, similar findings were seen (Figure 3).

Furthermore, as seen in Figure 4, cells that were sensitive or resistant to cisplatin and were transfected with HMGA2 siRNAs proliferated at a significantly slower rate than cells that were transfected with control siRNAs.

Given the notion that the HMGA2 protein plays a crucial role in the control of the cell cycle, we have investigated the potential function that *HMGA2* silencing may have in the control of cell cycle of EC cells. Therefore, EC cells that were sensitive to cisplatin and resistant to it were transfected with HMGA2 siRNAs or with scrambled control siRNAs. Following a 72-h growth period under normal culture conditions, the cells were examined by flow cytometry. The results of the flow cytometric examination demonstrated that all of the EC HMGA2-siRNAs transfected cells exhibited an arrest in the S phase when compared to the control cells (Figure 5, Figure S1). As in cells arrested in S phase, the expression of G2/M cyclins is not induced, we used real-time PCR to examine the expression of *CCNB1* (*Cyclin B1*) and *CDK1* in both cisplatin-sensitive and cisplatin-resistant GCT27 and EP2102 cells. Both genes were downregulated in every sample where *HMGA2* was silenced (Figure 6). These findings imply that HMGA2 depletion may hinder the progression of the cell cycle by lowering the expression of important G2/M transition regulators.

2.3 | EC Cell Apoptosis and Migration Depend on HMGA2 Expression

To examine further the function of HMGA2 in apoptotic cell death, we inhibited its expression in EP2102 and GCT27 cells using siRNAs. As demonstrated in Figure 7, the *HMGA2* silencing significantly induced programmed cell death in GCT27, GCT27 CisR, and in EP2102 cells. Subsequently, we analyzed the expression of apoptotic markers AKT and cleaved PARP by Western blot. As shown in Figure 8, these two proteins are modulated in apoptotic cells: AKT levels decrease [24], while cleaved PARP levels increase [25] in HMGA2-silenced cells.

In previous papers, evidences from our and others groups reported that HMGA2 promotes cancer cell migration [26]. Here we examine the cell migration of the HMGA2 siRNAs transfected cells in order to determine whether or not this phenomenon also occurs in EC cells. As expected, 48 h after plating, a substantial reduction in cell migration was seen in all EC cell lines in which *HMGA2* have been silenced (Figure 9).

3 | Materials and Methods

3.1 | Cells and Cell Culture

A patient who had not had treatment before gave rise to the EC cell line GCT27, whose cell biology characteristics have been documented [27]. As previously reported, GCT27cis-r cells were produced by persistently increasing cisplatin concentrations [28]. Francesc Viñals of The Catalan Institute of Oncology, Universitat de Barcelona-IDIBELL, Spain, generously donated paired cell lines. The Health Protection of Agency Culture Authentication Service in the United Kingdom verified the cell lines in 2012 [29]. An EC cell line called 2102EP was obtained from a primary human testicular teratocarcinoma [30]. 2102EP cells were cultured for an extended period of time at progressively higher cisplatin concentrations to produce the 2102EPcis-r subline [31]. By kind donation, Dr. Michael Höpfner of the University of Berlin, Germany, supplied paired 2102EP cell lines. In Dulbecco's high-glucose Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA), EC cell lines were cultivated. Furthermore, 10% fetal bovine serum (FBS) (GIBCO, Waltham, MA, USA), antibiotics, and L-glutamine (GIBCO) were added. Aurogene (Rome, Italy) provided 0.5X trypsin-EDTA for the harvesting of EC cell lines. Upon routine inspection, all cells were determined to be *Mycoplasma*-free.

3.2 | siRNAs and Transfections

Using the Neon Electroporation System (Invitrogen), siRNAs for *HMGA2* or the control scrambled siRNAs, which were acquired from IDT (Coralville, IA, USA), were transfected into human ECS cells in accordance with the manufacturer's instructions.

3.3 | Western Blot

Western blot analyses were performed as previously described [18, 32–34]. Antibodies against HMGA2 are polyclonal Ab raised against a synthetic peptide located in the NH2-terminal region. The primary antibodies used were as follows: anti-AKT1 (9272), anti-PARP (9542), and anti-cleaved PARP (asp214) (9541) from Cell Signaling, MA, USA. As a control for equal loading of protein lysates, blotted proteins were probed with antibodies

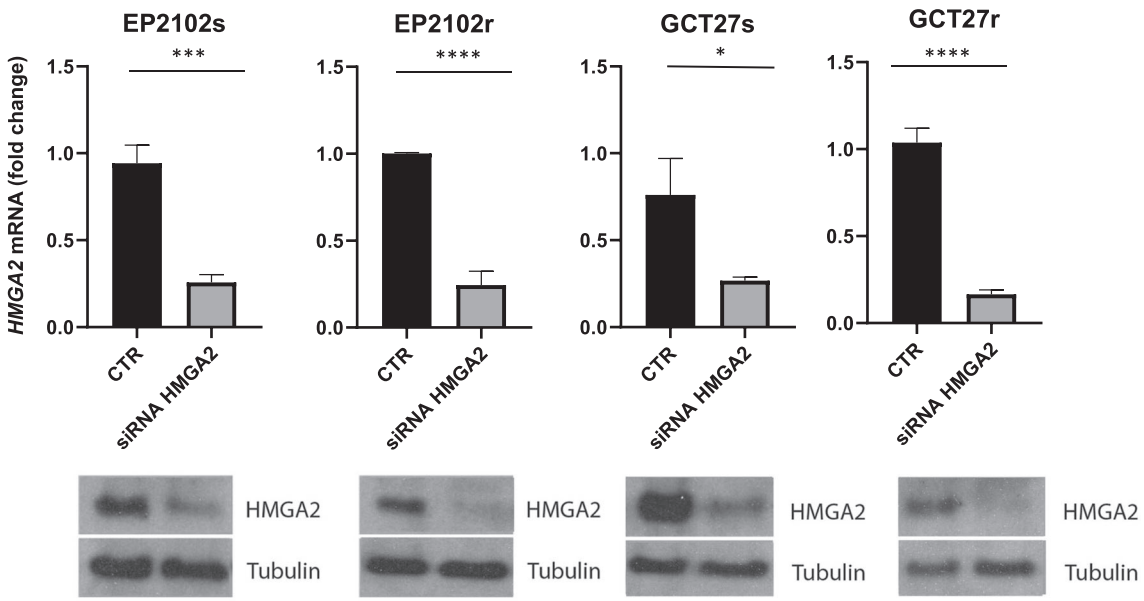

FIGURE 2 | *HMGA2* silencing in EP2102, GCT27 and their cisplatin resistant counterparts. (Upper panel) qRT-PCR analysis of *HMGA2* mRNA levels in EP2102s, EP2102r, GCT27s, and GCT27r cells transfected with the scrambled oligonucleotide or HMGA2-siRNA (lower panel). Western blot analysis of HMGA2 protein levels from the same samples shown in the upper panel. The results are reported as the mean of values. The error bars represent mean ± SD; $n = 3$; *$p < 0.005$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

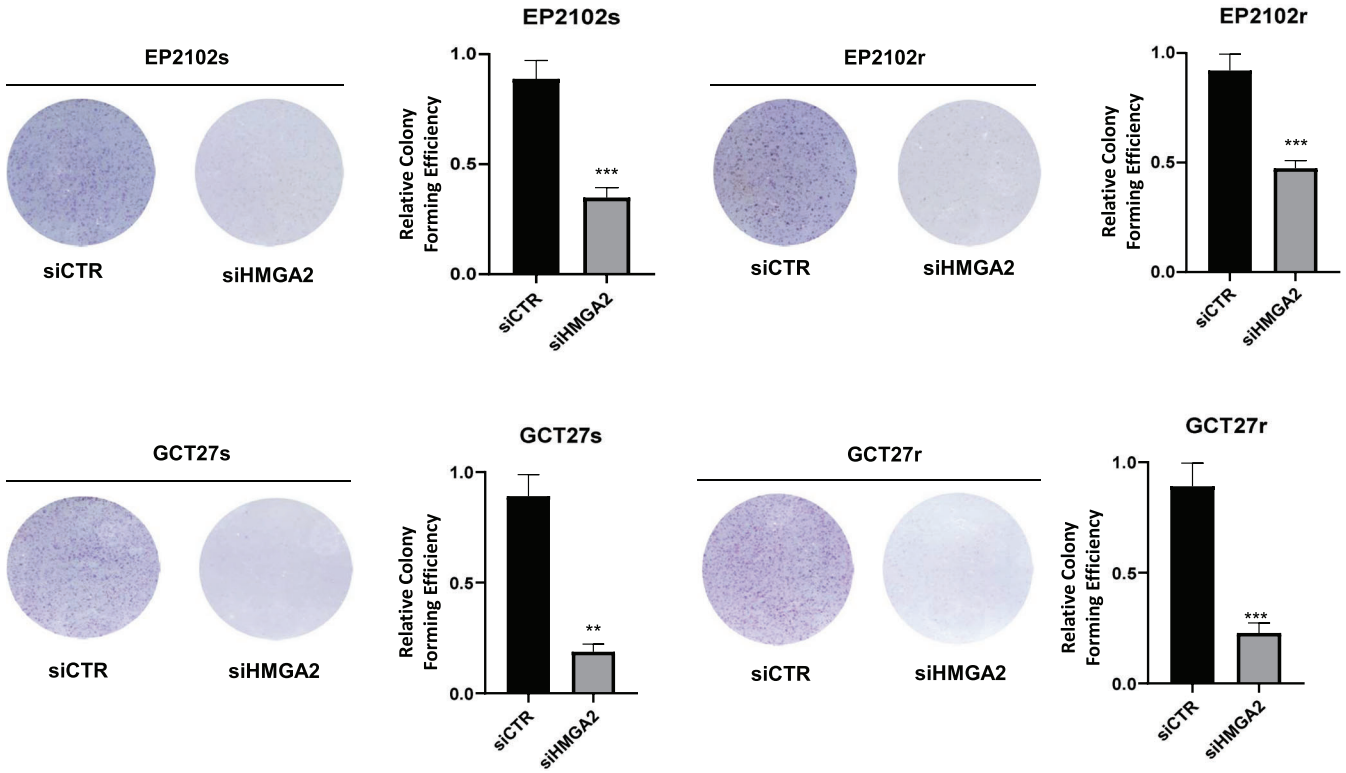

FIGURE 3 | Effect of HMGA2-silencing on EC cell colony assay. Representative images of colony assay carried out in EP2102s, EP2102r, GCT27s, and GCT27r cells transfected with the scrambled oligonucleotide or HMGA2-siRNA. After 96 h the cells were stained with crystal violet solution. Colony forming efficiency was calculated by crystal violet absorbance. The error bars represent mean ± SD; $n = 3$; *$p < 0.005$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

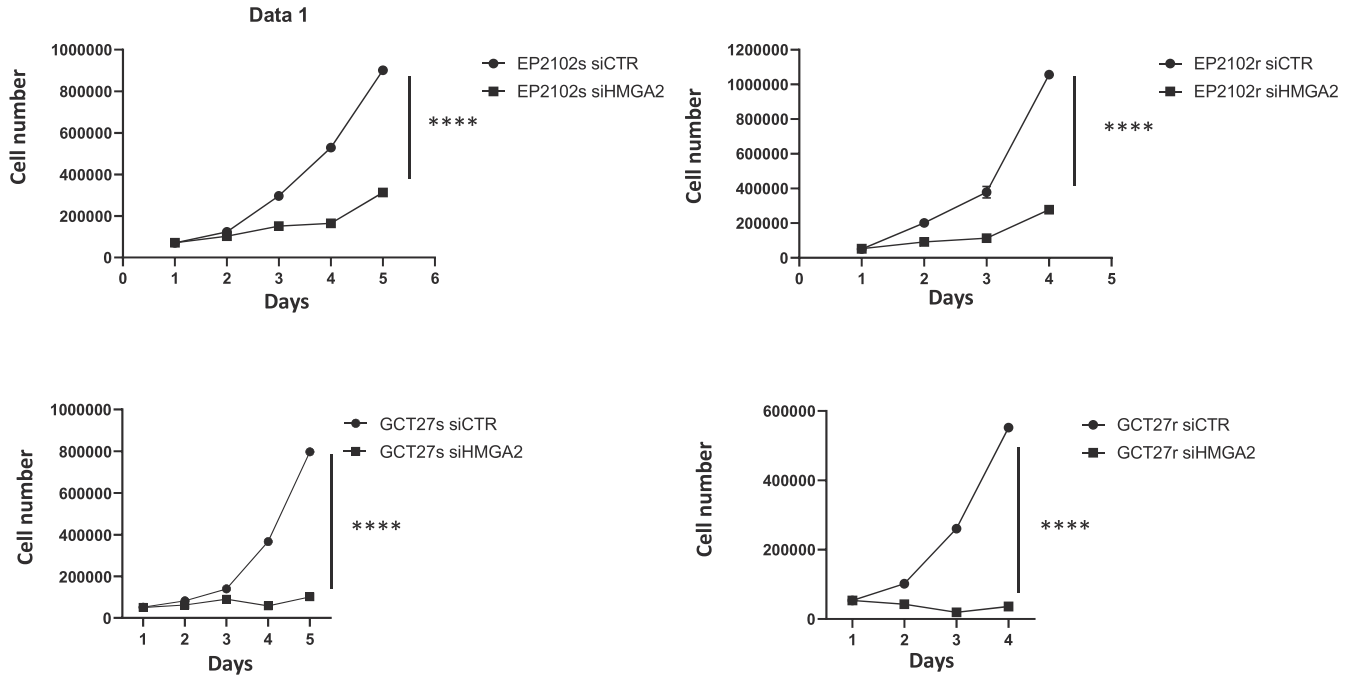

FIGURE 4 | HMGA2 has a key role on the proliferation of EC cells. EP2102s, EP2102r, GCT27s, and GCT27r cells proliferation was assayed in siHMGA2-transfected cells, compared with mock-transfected cells. Cell counting was performed up to the time point at which HMGA2 siRNA–treated cells were still growing, as beyond this point proliferation was no longer detectable. The error bars represent mean ± SD; $n = 3$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

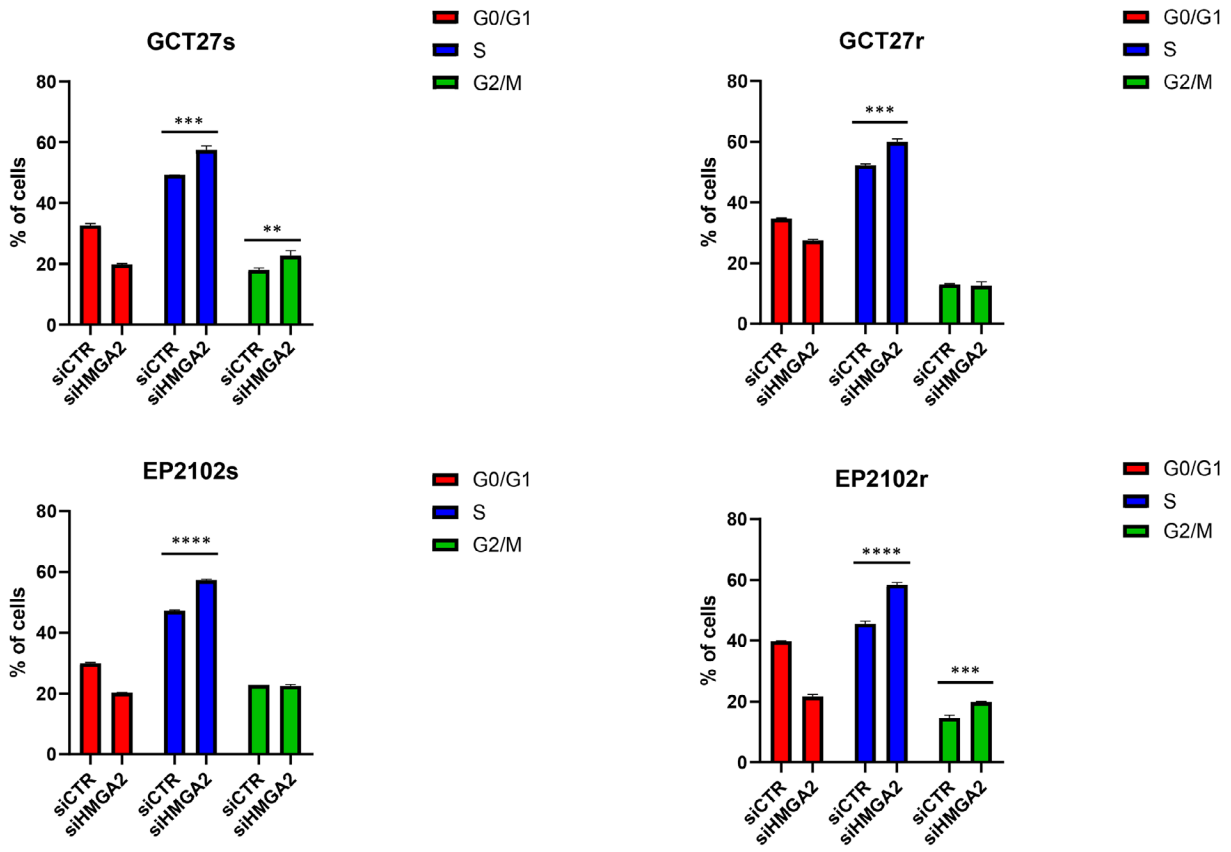

FIGURE 5 | *HMGA2* silencing impairs EC cell cycle progression. The DNA content of the transfected EC cells was analyzed by flow cytometry after propidium iodine staining. The results are reported as the mean of values. The error bars represent mean ± SD; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

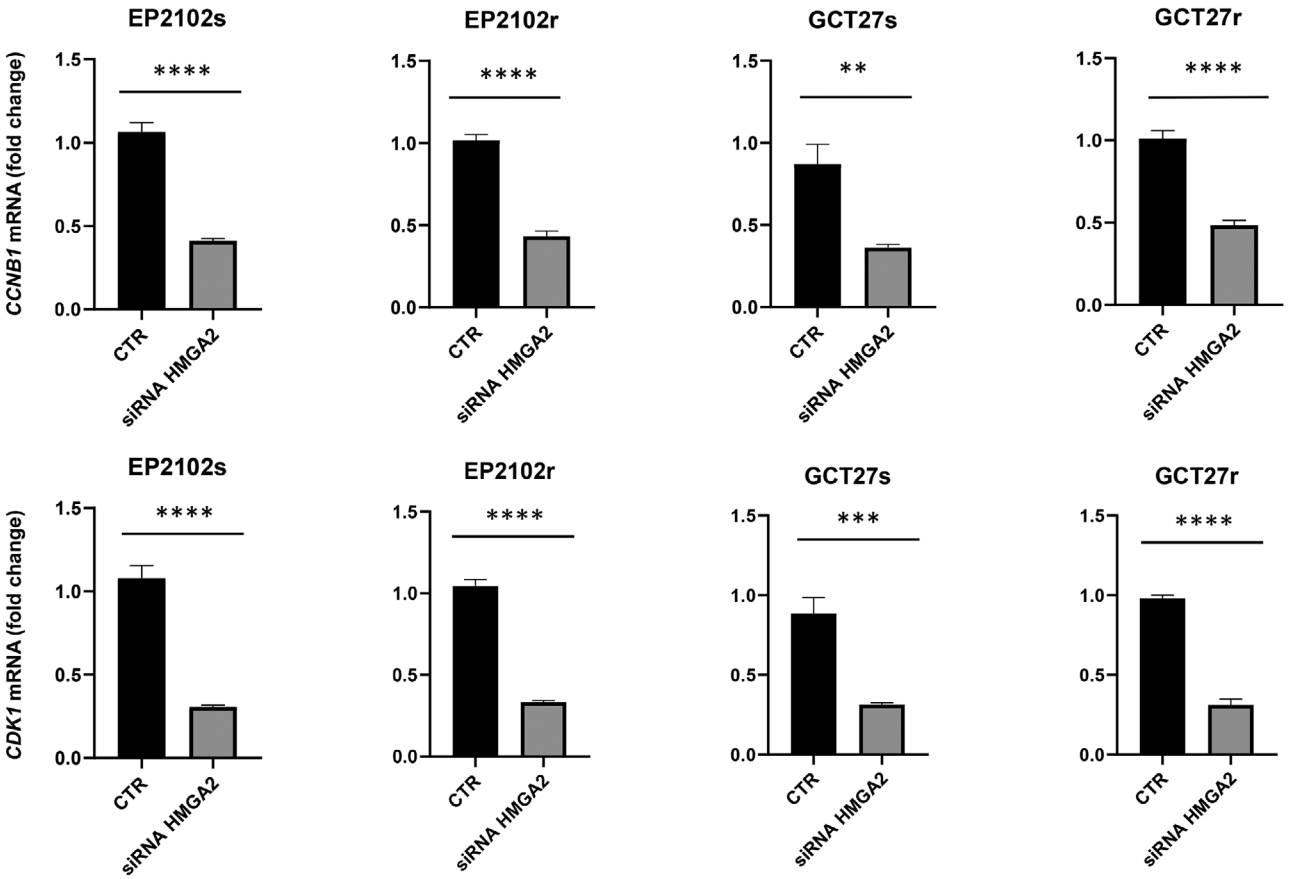

FIGURE 6 | *HMGA2* silencing reduces *CCNB1* and *CDK1* expression in cisplatin-sensitive and -resistant GCT27 and EP2102 cells. Cisplatin-sensitive and -resistant GCT27 and EP2102 cells, which display an S-phase arrest, were analyzed by real-time PCR for the expression of *CCNB1* (*Cyclin B1*) and *CDK1* after *HMGA2* silencing. Data are presented as mean ± SD; $n = 3$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

against GAPDH protein (sc-32233) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or β Tubulin protein (32-2600) (Thermo Fisher Scientific, MA, USA).

3.4 | Real Time PCR

Total RNA was extracted from cell lines (1×10^6 cells) using the Trizol reagent (Thermo Fisher Scientific, Massachusetts, USA). Each sample included 1 μg of total RNA, which was retrotranscribed into double-stranded cDNA using the Quanti-Tect Reverse Transcription Kit (Qiagen, Hilden, Germany). The CFX96 thermocycler (Bio-Rad, Hercules, CA, USA) was used for quantitative real-time PCR (qRT-PCR) experiments. 10 μL of 2X SYBR Green (Bio-Rad, CA, USA), 200 nM of each primer, and 20 ng of the previously generated cDNA were used for each PCR experiment [35]. Primer-BLAST software was used to create oligonucleotides that covered exon–exon junctions for qRT-PCR. Using primers acquired from Integrated DNA Technologies (San Diego, CA, USA), the relative gene expression was ascertained using the comparative C(T) approach, as previously described [36]. MCF7 cells were used as a *HMGA2*-negative control and HepG2 cells as a *HMGA2*-positive control in the PCR experiments.

HMGA2-forward 5′-CCCTCTAAAAGCAGCTCAAAAGA-3′;

HMGA2-reverse 5′-TGGTAGTAGATTGTCCCATTTCC-3′;

CCNB-forward 5′-CATGGTGCACTTTCCTCCTT-3′;

CCNB-reverse 5′-AGGTAATGTTGTAGAGTTGGTGTCC-3′;

CDK1-forward 5′-CCATGGGGATTCAGAAATTG-3′;

CDK1-reverse 5′-CCATTTTGCCAGAAATTCGT-3′;

GAPDH-forward 5′-ACAGAGTGAGCCCTTCTTCAA-3′;

GAPDH-reverse 5′-ATAGGAGTTGCGGGCAAAG-3′.

3.5 | Growth Curve and Colony Assay

Following the suppression of *HMGA2* expression by siRNAs transfection, the proliferation rate was assessed using a Burker hemocytometer chamber to evaluate cell growth. In brief, 3×10^4 cells were seeded in a 60 mm plate. To assess the rate of cell proliferation, cells were counted in triplicate using a Burker hemocytometer chamber for 5 days. Ep2102 and GCT27 cells, sensitive and resistant to cisplatin, were transfected with siRNAs for *HMGA2* and seeded in 60 mm dishes for colony tests. After 96 h, plates were fixed using a solution that included 20% methanol and 0.05% crystal violet.

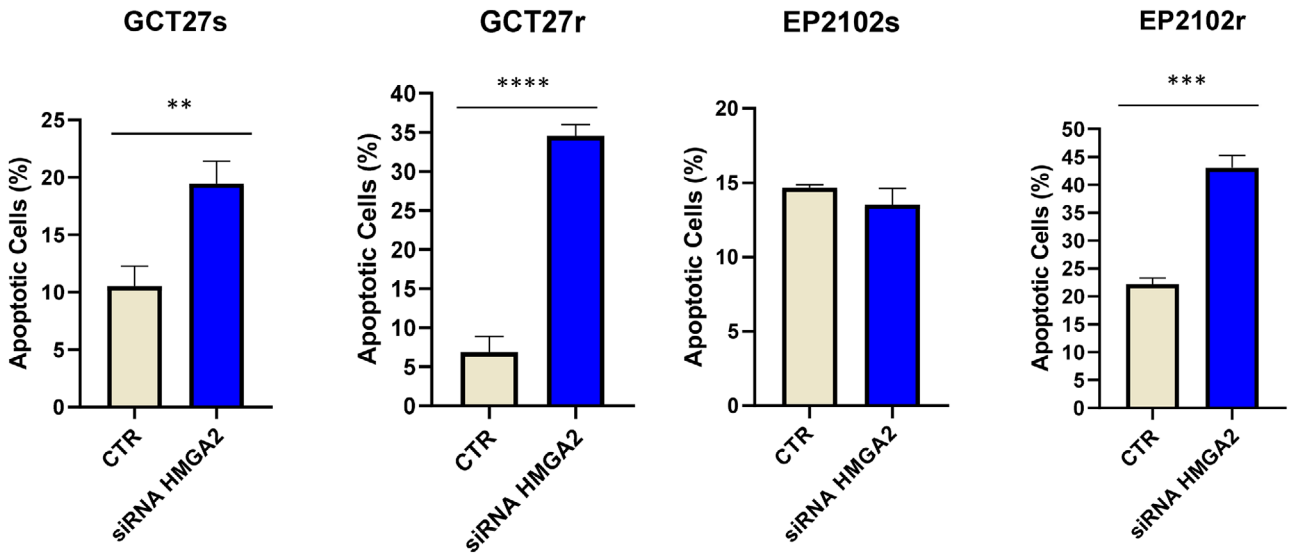

FIGURE 7 | Effects of *HMGA2* silencing on EC cells apoptosis. Quantification of EP2102s, EP2102r, GCT27s, and GCT27r Annexin V positive cells 72 h following HMGA2 siRNA transfection. Data are shown as mean ± SD; $n = 3$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

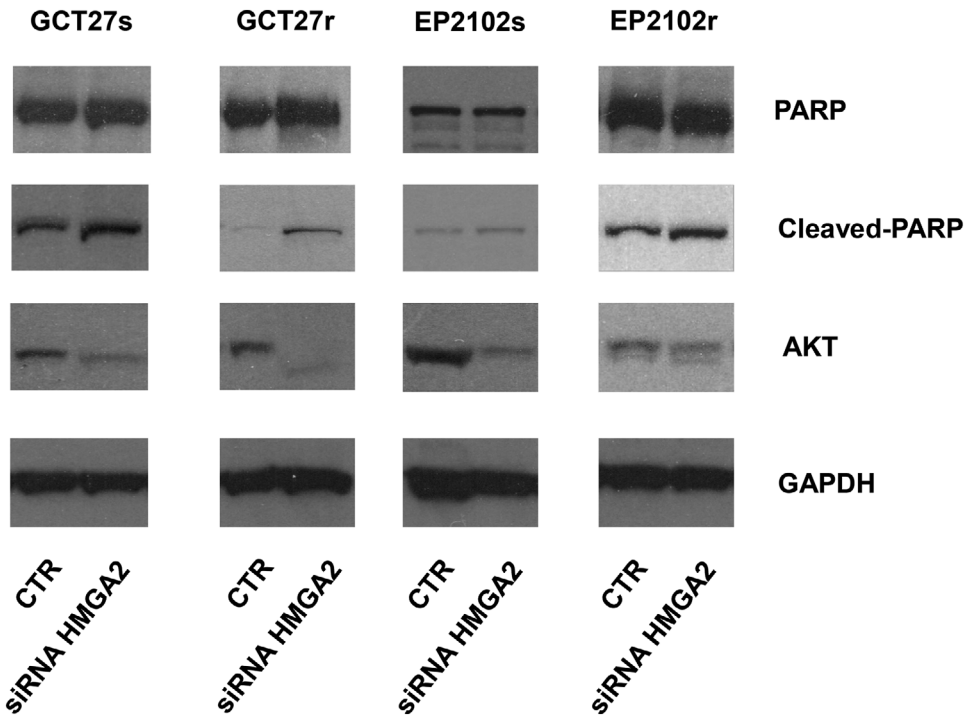

FIGURE 8 | *HMGA2* silencing reduces AKT expression and enhances cleaved PARP levels in cisplatin-sensitive and -resistant GCT27 and EP2102 cells. AKT1, PARP, and cleaved PARP protein levels in EP2102s, EP2102r, GCT27s, and GCT27r cells transfected with the scrambled oligonucleotide or HMGA2-siRNA. Fifty micrograms of total cellular lysate was loaded per lane in reducing conditions. GAPDH was used as protein loading control.

3.6 | Cell Cycle Analysis

Cell cycle distribution was investigated using fluorescence-activated cell sorting, and the data were interpreted using the free online software Floreada.io (https://floreada.io). Following 72 h from HMGA2 siRNAs transfection, EC cells were trypsinized, and centrifuged at 1500 *g* for 5 min. After that, the cells were frozen for the night at −20°C using 70% ethanol (Carlo Erba, Milan, Italy). Following their removal from the ethanol solution, the cells underwent two PBS washes (Sigma-Aldrich, Saint Louis, MO, USA). Approximately, 300 μL of a solution containing ribonuclease and propidium iodide (PI) dye (Thermo Fisher Scientific, MA, USA) was then added to the cells. The data was evaluated using the free online software Floreada.io, and the findings were shown as a percentage of cells in each cell cycle stage.

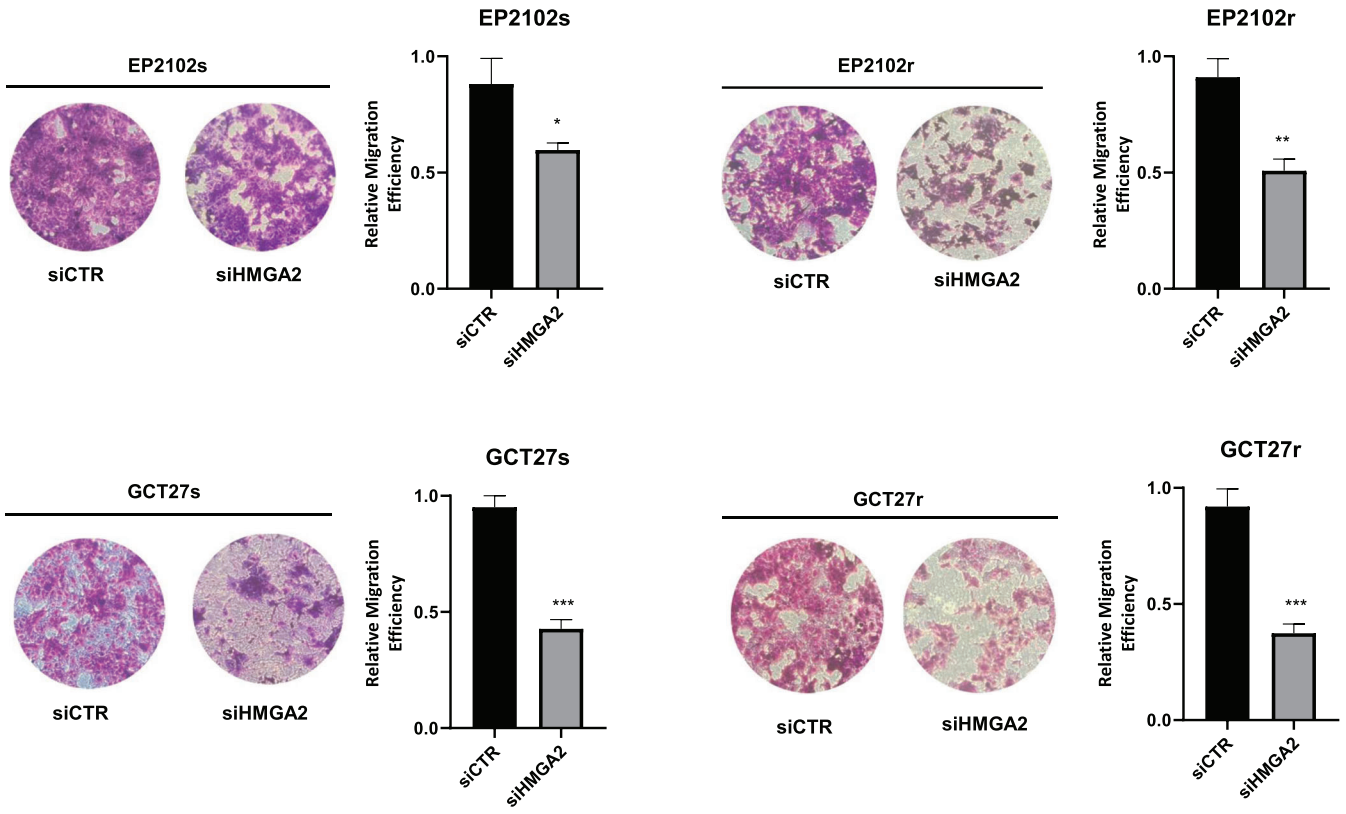

FIGURE 9 | *HMGA2* silencing reduces the migration of EC cells. EP2102s, EP2102r, GCT27s, and GCT27r cells were transfected with HMGA2 siRNA or with a control not targeting scrambled siRNA. Then, the cells were seeded on 5-mm pore size Transwell filters and allowed to migrate toward 10% fetal bovine serum. After 48 h, cells on the underside of the filters were fixed with crystal violet solution. After elution from transwell, it was quantified by reading its absorbance at 590 nm. Data are shown as mean ± SD; $n = 3$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (*t*-test).

3.7 | Annexin Test

Approximately, 1.5×10^5 cells transfected with siRNAs for *HMGA2* were resuspended in 2 mL of culture media and subsequently seeded in 6-well plates in order to measure apoptosis. In accordance with the manufacturer's instructions, after 72 h cells were exposed to FITC-Annexin V and PI (BD Biosciences, San Jose, CA, USA). This enables us to differentiate between cells that are Annexin V-positive during early apoptosis and those that are Annexin V- and PI-positive during late apoptosis. The total of the early and late apoptotic cells was the outcome. Using FL1 for Annexin V and FL2 for PI, cells were examined using flow cytometry. Cell Quest Pro software was utilized to evaluate data obtained from flow cytometry (FACScan; BD Biosciences).

3.8 | Cell Migration Assay

As previously mentioned [37], the transwell migration experiment was carried out. In short, 0.2 mL of serum-free medium was used to seed 3×10^4 cells in the upper chamber, while 0.5 mL of complete medium was introduced as a chemoattractant in the lower chamber. The migrating cells were fixed and stained with crystal violet solution (crystal violet 0.05%, methanol 20%) following a 48-h incubation period.

After de-staining the chamber with PBS-0.1% SDS solution, the crystal violet was measured at 590 nm using a microplate reader (LX800, Universal Microplate Reader, BioTek Instruments).

In addition, 3×10^4 cells were seeded in a 96-well plate to normalize the amount of cells. Three hours later, the absorbance at 490 nm was measured using a cell titer (Promega) in a microplate reader (LX800, BioTek Instruments). The crystal violet measurements were normalized to cell titer values in order to achieve the results.

3.9 | Analysis of Statistics

The statistical analyses were carried out using GraphPad Prism. The statistical significance of the data collected was evaluated using the unpaired *t*-test. The significance level for each experiment was set at $p < 0.05$. The data's standard deviation (SD) and mean values are displayed.

3.10 | Data From the Cancer Genome Atlas

Publicly available transcriptomic data from The Cancer Genome Atlas (TCGA) TGCT cohort were used to evaluate *HMGA2* mRNA expression across tumor subtypes. Normal testicular tissue samples ($n = 60$) and tumor samples comprising seminoma

(Sem; $n = 64$), embryonal carcinoma (EC; $n = 24$), yolk sac tumor (YST; $n = 4$), and teratoma (Ter; $n = 8$) were included in the analysis. Gene expression data were obtained as normalized RNA sequencing values (log10-transformed) generated using the TCGA standardized bioinformatics pipeline. *HMGA2* expression levels were extracted and grouped according to histopathological subtype. Data visualization was performed using scatter or dot plots, with each point representing an individual sample and central tendency indicated as appropriate.

4 | Discussion

Our results show that HMGA2 is essential for several tumorigenic processes in EC cells, such as migration, apoptosis, cell cycle control, and proliferation, with comparable effects observed in both cisplatin-sensitive and -resistant models.

However, the literature presents partially contrasting evidence regarding *HMGA2* expression in EC. While Kloth et al. [38] reported no significant overexpression of *HMGA2* in ECs, Murray et al. [39] found marked *HMGA2* upregulation in a subset of ECs, suggesting that *HMGA2* expression may depend on specific molecular or histological subtypes. Our findings are consistent with the latter observation, reinforcing the view that HMGA2 contributes to tumor aggressiveness and chemoresistance in EC.

Our findings demonstrate that *HMGA2* silencing significantly impairs proliferation of both cisplatin-sensitive and -resistant EC cells, as demonstrated by colony formation assays and growth curves. This is in line with earlier reports that implicate HMGA2 as a promoter of cell proliferation in various cancers [17, 26]. This implies that HMGA2 is necessary for long-term cell proliferation regardless of cisplatin sensitivity. Consistently, *HMGA2* knockdown produces cell cycle arrest in the S phase, according to flow cytometric investigations, suggesting a function in regulating DNA replication and cell cycle progression, most likely via transcriptional regulation of cyclins and CDKs [16, 40].

HMGA2 knockdown increased apoptosis in addition to inhibiting proliferation, especially in GCT27 and cisplatin-resistant cells, highlighting its role in promoting tumor cell survival. This is consistent with findings from other tumor models, such as ovarian cancer [41] and pancreatic cancer [42], where HMGA2 has been demonstrated to suppress apoptotic pathways by altering the expression of anti-apoptotic genes including *survivin* and *BCL-2*. Crucially, HMGA2-silenced 2102EP cells showed a significant suppression of cell proliferation without an increase in cell death, suggesting a cytostatic rather than cytotoxic effect. As it indicates a context-dependent reliance on HMGA2 for survival in the cisplatin-resistant phenotype, we think the selective induction of apoptosis in resistant cells is especially pertinent. We are currently looking into the pathways that could explain the different responses between parental and resistant cells, even though the exact molecular mechanism underlying this difference is still unclear. The selective vulnerability of resistant cells may reflect context-dependent dependencies on DNA damage response, replication stress tolerance, or apoptotic thresholds, which warrant further investigation.

Our findings clearly show that HMGA2 is still necessary for the growth and survival of cisplatin-sensitive and -resistant EC cells, even though they do not show a distinctive role for HMGA2 in the development of cisplatin resistance. From a translational perspective, this discovery is very significant because it demonstrates that HMGA2 inhibition works even in resistant cells, for which there are currently few available treatment alternatives. Therefore, the finding that *HMGA2* silencing causes apoptosis and growth arrest in resistant clone points to a possible therapeutic vulnerability that endures after chemoresistance develops. Instead of serving as a direct facilitator of resistance, HMGA2 seems to maintain important oncogenic characteristics shared by resistant and sensitive ECs, preserving their malignant phenotype regardless of the cisplatin response. Therefore, HMGA2 targeting may offer a useful tactic to hinder tumor growth in primary and refractory ECs.

HMGA2 is known to promote cytoskeletal reorganization and the epithelial–mesenchymal transition (EMT), which is consistent with the significant decrease in cell migration seen following *HMGA2* silencing [43]. Given that invasion and metastasis, two features of advanced illness that rely on migratory potential, are hallmarks of EC, this observation is especially pertinent.

When combined, these findings provide compelling evidence for a paradigm in which HMGA2 maintains malignant phenotypes and resistance to treatment in EC. Crucially, the data suggests that cisplatin-resistant cells need its expression to survive and proliferate, indicating that targeting HMGA2 may give a synthetic fatal approach in resistant cancers. These results offer a strong justification for the creation of HMGA2-targeted strategies, such as siRNA-based treatments, small molecule inhibitors, or antisense oligonucleotides, given the pressing need for efficient treatments in advanced or recurrent EC. While our data highlight HMGA2 as a potential vulnerability in resistant EC cells, the exact pathways mediating this effect are still unknown, and preclinical in vivo models will be necessary to confirm translational applicability.

Future research ought to assess HMGA2 inhibition in in vivo models of chemoresistant EC and investigate combination approaches with immune checkpoint inhibitors or DNA-damaging drugs, as HMGA2 modulation may improve response by boosting tumor immunogenicity and death.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Flow-cytometry gating strategy. Gating hierarchy of a representative sample (EPS siA2). (a) in SSC-A versus FSC-A, identification of the cloud of cell-like events; (b) in FSC-H versus FSC-A, identification of singlets (single cell signals); (c) PE-A histogram shows the cell-cycle profile