HMGA1 Is a Molecular Determinant of Chemoresistance to Gemcitabine in Pancreatic Adenocarcinoma

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Abstract

Purpose: HMGA1 proteins are architectural transcription factors that are overexpressed by pancreatic adenocarcinomas. We previously have shown that RNA interference targeting the HMGA1 gene may represent a potential chemosensitizing strategy in pancreatic adenocarcinoma cells. In this study, we tested the hypothesis that HMGA1 promotes chemoresistance to gemcitabine in pancreatic cancer cells.

Experimental Design and Results: Stable short hairpin RNA–mediated HMGA1 silencing in BxPC3 and MiaPaCa2 cells promoted chemosensitivity to gemcitabine, with reductions in gemcitabine IC_{50} and increases in gemcitabine-induced apoptosis and caspase-3 activation. In contrast, forced HMGA1 overexpression in MiaPaCa2 cells promoted chemoresistance to gemcitabine, with increases in gemcitabine IC_{50} and reductions in gemcitabine-induced apoptosis and caspase-3 activation. Dominant negative Akt abrogated HMGA1 overexpression–induced increases in chemoresistance to gemcitabine. Finally, HMGA1 silencing promoted chemosensitivity to gemcitabine in vivo in a nude mouse xenograft model of pancreatic adenocarcinoma.

Conclusion: Our findings suggest that HMGA1 promotes chemoresistance to gemcitabine through an Akt-dependent mechanism. Targeted therapies directed at HMGA1 represent a potential strategy for ameliorating chemoresistance in pancreatic adenocarcinoma.

Materials and Methods

Cells and cell culture. MiaPaCa2 and PANC1 human pancreatic ducal adenocarcinoma cells were obtained from American Type Culture Collection. Cells were maintained in DMEM containing 10% fetal bovine serum (Life Technologies) and incubated in a humidified atmosphere.

Overall prognosis for patients diagnosed with pancreatic adenocarcinoma remains dismal, with 5-year survival rates averaging <5% (1). At the time of diagnosis, most patients have locally advanced or metastatic disease precluding surgical resection (2). First-line therapy for most patients with advanced pancreatic cancer is based on the nucleoside analogue gemcitabine. However, the clinical response rate to gemcitabine remains modest, in part due to the profound chemoresistance inherent in pancreatic cancer cells. Therefore, characterization of mechanisms mediating chemoresistance in pancreatic adenocarcinoma is an important priority.

The human HMGA1 gene, located on chromosomal locus 6p21, encodes two HMGA1 splice variants (HMGA1a and HMGA1b; ref. 3). These HMGA1 proteins are architectural transcription factors that form stereospecific, multiprotein complexes termed “enhancosomes” on the promoter/enhancer regions of genes they regulate (4–6). Each HMGA1 protein has three AT-hook domains that can bind to the minor groove of AT-rich DNA sequences (4, 7). HMGA1 proteins are overexpressed in a wide range of human cancers, including pancreatic adenocarcinoma (8–15). Further, tumoral HMGA1 overexpression has been reported to be associated with poor prognosis in cancer patients (9, 10, 16).

Our group has previously reported the important roles played by HMGA1 in mediating cellular invasiveness and metastatic potential of pancreatic adenocarcinoma cells (17). Overexpression of HMGA1 promotes cellular invasion in vitro whereas posttranscriptional silencing of HMGA1 inhibits the ability of pancreatic adenocarcinoma cells to form metastases in vivo. Furthermore, we showed that overexpression of HMGA1 is associated with increased resistance to apoptosis under anchorage-independent culture (“anoikis resistance”; ref. 18). In these studies, we have reported that Akt is a key downstream effector of HMGA1-dependent signaling in pancreatic cancer (17). Given the central role of Akt in mediating chemoresistance to gemcitabine (19–21), we hypothesized that HMGA1 would promote chemoresistance to this agent through an Akt-dependent mechanism. In this study, we have confirmed this hypothesis to be correct, and in addition, we have shown that targeted posttranscriptional silencing of HMGA1 promotes chemosensitivity in vivo. In conclusion, our findings are of particular clinical importance as they suggest that targeted therapies directed against HMGA1 may ameliorate chemoresistance to gemcitabine.
Reagents and dominant negative Akt constructs. Anti-HMGA1, anti–lamin B1, anti–phospho-Akt (Ser473), anti-Akt1, and anti–hemagglutinin antibodies were obtained from Santa Cruz Biotechnology. Hemagglutinin-tagged dominant negative Akt adenovirus (Ad-DN-Akt) and its control adenovirus (Ad-CMV-Null) were purchased from Harvard Bioscience. 

Lentivirus-mediated HMGA1 RNA interference. Hairpin RNA interference plasmids (pLKO.1-HMGA1, TRCN00000018949) were obtained from The RNAi Core (Simpson-Archer). The sequences of short hairpin RNA targeting the human HMGA1 gene (Genbank accession no. NM_000131) was 5' CACACTCTCGAGGAACTAGGAA-3' (shHMGA1 targeting coding region positions 446-466 of HMGA1 mRNA transcript variant 2). The control pLKO.1 plasmid, which has a scrambled nontargeting short-hairpin RNA sequence, was obtained from Addgene (22). High-titer lentivirus expressing shHMGA1 and control short hairpin RNA (shRNA) were generated from five-plasmid transfection into 293T cells. Helper plasmids were pHDM-Hgpm2, pMD-tat, pRC/CMV-rev, and pCMV-VSV-G obtained from Harvard Gene Therapy Initiative (Harvard Medical School, Boston, MA). The lentiviral transfer vectors were either control pLKO.1 (nontargeting shRNA sequence) or shHMGA1 plasmids as described above. Virus preparations were concentrated by ultracentrifuge and titered by Southern blotting. We achieved titers of 7.2 × 10^8/mL for control shRNA lentivirus and 2.5 × 10^9/mL for shHMGA1 lentivirus. Pooled stable transfectants were developed following infection of lentivirus at multiplicity of infection of 10 for 48 hours, and stable selection in puromycin was achieved as described above.

Expression vector and transfection. The HMGA1 coding sequence was PCR amplified from IMAGE clone 5399570 (Genbank accession no. BC063434) using gene-specific primers modified to include the appropriate restriction sites at their 5' end. The primers used were as follows: forward, 5'-TTTGTATATCATGTCAGTCTCGAGAGCAAC-3' and backward, 5'-TTTGTCTCTTACTGTATGTCAGTCTCGAGAGCAAC-3'. Purified PCR products were digested with EcoRV and EcoRI, before ligation into a EcoRV/EcoRI-digested pIRES-puro3 vector (Clontech). The expression plasmids were named pIRES-HMGA1. MiaPaCa2 cells were transfected with pIRES-HMGA1 or empty pIRES-puro3 vector (Clontech), using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's protocol. Stable clones were selected by exposure to incrementally increasing concentrations of puromycin (Invivogen), isolated using cloning cylinders, and maintained in medium containing 3 μg/mL puromycin (Invivogen). Clones pIRES-HMGA1.1 and pIRES-HMGA1.2, which expressed the highest levels of HMGA1, were used for further studies.

Western blotting. Cells were harvested and rinsed twice with PBS. Total cell extracts were prepared with Phosphosafe lysis buffer (Novagen). Nuclear extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents based on the manufacturer’s instructions (Pierce). Protein concentration was measured using the BCA assay kit (Sigma). Cellular protein was subjected to 10% SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membranes (Invitrogen). After blocking with PBS containing 3% bovine serum albumin for 1 hour at room temperature, membranes were incubated with 3 to 5 μg/mL antibody in PBS containing 0.1% Tween 20 overnight at 4°C. Chemoluminescence detection (Amer sham Biosciences) was done in accordance with the manufacturer’s instructions. The densitometric signal was quantified using ImagePro Plus software version 4.0 (Media Cybernetics).

Cytotoxicity assay. Gemcitabine-induced cytotoxicity was quantified by a MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay (CellTiter 96, Promega). Cells were seeded into 96-well plates at 5 × 10^4 per well and allowed to adhere overnight. Cell viability was determined after 48 to 72 hours in presence or absence of 0 to 10 μmol/L gemcitabine. Plates were read with the use of the SpectraMax M5 microplate spectrophotometer (Molecular Devices) at a wavelength of 490 nm. IC50 values were calculated. At identical time points, cell counting was done. Viable cells, identified by trypan blue exclusion, were counted using a Neubauer hemocytometer (Hauser Scientific). Cell counting confirmed MTS results.

Apoptosis assay. After exposure to gemcitabine (1 μmol/L) for up to 24 hours, 1 × 10^6 cells were washed, trypsinized, and resuspended in 0.5 mL of PBS containing 2% fetal bovine serum and 0.1 μmol/L EDTA. Apoptosis staining was done using 1 μmol/L YO-PRO-1 and propidium iodide (Vybrant Apoptosis Assay Kit 4; Molecular Probes). Cells were then analyzed by flow cytometry (FACScan; Becton, Dickinson), measuring fluorescence emission at 530 and 575 nm. Cells stained with the green fluorescent dye YO-PRO-1 were counted as apoptotic; necrotic cells were stained with propidium iodide. The number of apoptotic cells was divided by the total number of cells (minimum of 10^4 cells) to calculate the apoptotic fraction.

Fluorescent caspase profiling. Whole-cell lysates were assayed for caspase-3 activity using the BD ApoAlert Caspase Assay Plate (BD Biosciences) according to the manufacturer’s instructions. Plates were read (excitation, 360 nm; emission, 480 nm) using SpectraMax M5 microplate reader in fluorescence mode (Molecular Devices).

Nude mouse subcutaneous xenograft model. Male athymic nu/nu mice 5 weeks of age, weighing 20 to 22 g, and specific pathogen-free were obtained from Harlan Sprague-Dawley. Mice were housed in microisolator cages in a pathogen-free facility with 12 hours light-dark cycles. Animals were observed for signs of tumor growth, activity, feeding, and pain in accordance with the guidelines of the Harvard Medical Area Standing Committee on Animals. To determine the effect of HMGA1 gene silencing on in vivo chemosensitivity, 2 × 10^6 MiaPaCa cells stably expressing control or HMGA1 shRNA (shHMGA1.1 sequence, lentiviral transduction) were s.c. implanted in nude mice. Mice were anesthetized with i.p. ketamine (200 mg/kg) and xylazine (10 mg/kg) and inoculated with 2 × 10^6 cells in 100 μL of PBS s.c. Gemcitabine administration was commenced 14 days after implantation when tumors were ~50 mm^3 in volume. Mice received gemcitabine (150 mg/kg) in 100 μL of PBS vehicle by twice-weekly i.p. injection. Tumor dimensions were measured weekly using micrometer calipers. Tumor volumes were calculated using the following formula: volume = 1/2 a × b^2, where a and b represent the larger and smaller tumor diameters, respectively. After 6 weeks of gemcitabine administration and 4 days after final gemcitabine injection, necropsy was done, and the primary tumor was excised, formalin-fixed, and paraffin embedded.

Immunohistochemistry. Tumor sections (5 μm) were deparaffinized, rehydrated through graded alcohol, and processed using a streptavidin-peroxidase complex method. Immunohistochemistry was performed using the following antibodies: anti–HMGA1, anti–K-ras, and anti–bcl-2. Tissue sections were deparaffinized, rehydrated through graded alcohol, and processed using the streptavidin-peroxidase complex method. Immunohistochemistry was performed using the following antibodies: anti–HMGA1, anti–K-ras, and anti–bcl-2. Tissue sections were incubated with rabbit anti-HMGA1 antibody (1:200 dilution; Santa Cruz Biotechnology) and incubated with biotin-peroxidase complex method. Sections were incubated with anti–HMGA1, anti–K-ras, and anti–bcl-2 antibodies, respectively. After 6 weeks of gemcitabine administration and 4 days after final gemcitabine injection, necropsy was done, and the primary tumor was excised, formalin-fixed, and paraffin embedded.

Statistical analysis. Differences between groups were analyzed using Student’s t test, multifactorial ANOVA of initial measurements, and Mann-Whitney U test, for nonparametric data, as appropriate, using Statistica 5.5 software (StatSoft, Inc.). In cases in which averages were normalized to controls, the SDs of each nominator and denominator were taken into account in calculating the final P. P < 0.05 was considered statistically significant.
Results

HMGA1 is a molecular determinant of chemoresistance to gemcitabine in pancreatic adenocarcinoma cells. Lentivirus-mediated HMGA1 silencing resulted in a marked increase in chemosensitivity to gemcitabine in BxPC3 cells (in which we achieved almost 90% silencing of HMGA1 protein expression; Fig. 1A), with ~4-fold reductions in IC_{50} to gemcitabine (mean IC_{50}, control shRNA versus shHMGA1; 50 versus 12 nmol/L, \( P = 0.001 \); Fig. 1C and D). Interestingly, BxPC3 cells in which HMGA1 had been silenced developed spiculated morphology on exposure to 1 \( \mu \)mol/L gemcitabine for 48 hours, whereas control cells did not (Fig. 1B). In our previous study, we achieved up to 90% silencing of HMGA1 expression in MiaPaCa2 cells using lentivirus-expressing shRNA with the same target sequence (23). In the current study, we achieved a similar degree of HMGA1 knockdown in MiaPaCa2 cells (data not shown). Lentivirus-mediated HMGA1 silencing had similar effects on MiaPaCa2 cells, with shifting of the gemcitabine IC_{50} curve to the left (Fig. 2B) and ~2-fold reductions on IC_{50} (mean IC_{50}, control shRNA versus shHMGA1; 60 versus 30 nmol/L, \( P = 0.001 \); Fig. 2D).

We then tested the effect of forced overexpression of HMGA1 on cellular chemoresistance to gemcitabine. MiaPaCa2 cells (which have low inherent expression of HMGA1) were stably transfected with the pIRES-HMGA1 vector, as described in Materials and Methods. We selected two transfectant clones with highest expression levels of HMGA1 (pIRES-HMGA1.1 and pIRES-HMGA1.2). In our previous studies, we have characterized the pIRES-HMGA1.1 and pIRES-HMGA1.2 clones and verified their overexpression of HMGA1 by Western analysis (17, 18). pIRES-HMGA1.1 and pIRES-HMGA1.2 clones overexpress HMGA1 by 4-fold and 3.5-fold, respectively, when compared with empty pIRES-puro3 transfectants (data not shown). Overexpression of HMGA1 resulted in significant increases in chemoresistance to gemcitabine, with increases in IC_{50} to gemcitabine (Fig. 2D) and shifting of the IC_{50} curves to the right for both pIRES-HMGA1.1 and pIRES-HMGA1.2 clones (Fig. 2C). The mean IC_{50} for pIRES-HMGA1.1 and pIRES-HMGA1.2 were 130 and 100 nmol/L, respectively (\( P = 0.003 \) and \( P = 0.006 \), respectively, versus empty pIRES-puro3 controls). Notably, pIRES-HMGA1.1 consistently overexpressed HMGA1 to a greater degree than pIRES-HMGA1.2; this greater HMGA1 overexpression was associated with a higher mean IC_{50} value. Forced HMGA1 overexpression enhanced the viability of cells exposed to 1 \( \mu \)mol/L of gemcitabine for 72 hours, whereas HGMA1 silencing was associated with the opposite effect (Fig. 2A).

HMGA1 expression status modulates gemcitabine-induced apoptosis and caspase-3 activation. Following exposure to 1 \( \mu \)mol/L gemcitabine for 24 hours, cells were subjected to flow cytometric quantitation of apoptosis and fluorometric caspase-3 profiling. Lentivirus-mediated HMGA1 silencing was associated with increases in chemosensitivity to gemcitabine and caspase-3 activation (Fig. 3A) and caspase-3 activation in both BxPC3 and MiaPaCa2 cells (Fig. 3C). In contrast, HMGA1 overexpression was

Fig. 1. A, following the generation of high-titer lentivirus particles carrying shHMGA1, BxPC3 was transduced with lentivirus at multiplicity of infection of 10 and stable transfectants were developed following selection with puromycin. Robust suppression of HMGA1 was achieved using lentivirus with a high degree of silencing of HMGA1. In BxPC3 cells, lentivirus-mediated shHMGA1 achieved almost complete silencing of HMGA1. Controls were stable transfectants developed using lentivirus carrying scrambled, nontargeting shRNA. B, the effects of lentivirus-mediated HMGA1 silencing on chemosensitivity to gemcitabine were assessed. When BxPC3 cells in which HMGA1 had been silenced were exposed to 1 \( \mu \)mol/L gemcitabine for 48 h, they adopted a less healthy, spiculated morphology compared with control cells. Photomicrographs were taken using an inverted microscope at \( \times 40 \) magnification. C, survival curves following exposure to 0 to 10 \( \mu \)mol/L gemcitabine were analyzed following MTS assay. Lentivirus-mediated stable HMGA1 silencing in BxPC3 cells shifted the survival curve to the left, indicating an increase in chemosensitivity to gemcitabine, when compared with the controls. Correspondingly, there was a 4-fold reduction in the IC_{50} to gemcitabine with silencing of HMGA1 when compared with the controls. * \( P = 0.001 \) versus control shRNA.
associated with reductions in gemcitabine-induced apoptosis and in caspase-3 activation (Fig. 3B and D).

**HMGA1-induced chemoresistance to gemcitabine is dependent on Akt signaling.** We have previously reported that Akt is a downstream effector of HMGA1 (18, 23). We have found that HMGA1 silencing is associated with reductions in Akt phosphorylation (a marker of Akt activation), whereas forced HMGA1 overexpression is associated with increases in Akt kinase activity and in Akt phosphorylation (17, 18). Given the importance of the phosphatidylinositol 3-kinase/Akt pathway in ant apoptotic signaling, particularly in the context of chemoresistance, we sought to determine if chemoresistance to gemcitabine-induced HMGA1 overexpression is Akt dependent. Each of pIRES-HMGA1.1 and pIRES-HMGA1.2 clones were transduced with dominant negative Akt adenovirus at multiplicity of infection of 10. The efficiency of transduction and expression of dominant negative Akt were confirmed by immunoblotting for the hemagglutinin tag of the dominant negative Akt construct (Fig. 4). We next assessed the effects of dominant negative Akt on chemosensitivity to gemcitabine in each HMGA1-overexpressing clone. Dominant negative Akt was found to reverse the chemoresistance induced by HMGA1 overexpression, with reductions of IC50 to gemcitabine in both pIRES-HMGA1.1 and pIRES-HMGA1.2 clones. As such, HMGA1 overexpression–induced chemoresistance is dependent on Akt signaling.

**HMGA1 silencing promotes chemosensitivity to gemcitabine in vivo.** BxPC3 cells in which HMGA1 had been silenced through stable lentiviral shRNA-mediated RNAi or control BxPC3 cells transduced with lentivirus carrying nontargeting shRNA were s.c. implanted into nude mice. Once the resulting xenograft tumors had grown to reach ~50 mm3 in diameter, a 6-week course of gemcitabine administration was initiated. Tumors derived from BxPC3 cells in which HMGA1 had been silenced (n = 8 animals) regressed during the treatment period, whereas tumors derived from control cells (n = 8 animals) continued to...
grow during the treatment period (Fig. 5A–C). Stable suppression of HMGA1 expression in tumors derived from shHMGA1 transfectants was confirmed on Western blotting of nuclear extracts of tumor homogenates (Fig. 6A) and on immunohistochemical analysis of xenografts harvested at the end of the study period (Fig. 6B). TUNEL staining revealed significantly higher apoptotic index in shHMGA1 transfectant–derived tumors than in control cell–derived tumors (Fig. 6B and C).
Discussion

HMGA1 proteins are overexpressed in a wide range of human cancers, including pancreatic adenocarcinoma (8). Experimental data implicating biologically important roles for HMGA1 in cancer pathogenesis are rapidly accumulating (24, 25). Our study provides the first data suggesting that HMGA1 may mediate a critical feature of malignant phenotype: chemoresistance. First, our findings show that forced HMGA1 overexpression promotes chemoresistance to gemcitabine in pancreatic cancer cells in vitro, whereas HMGA1 silencing promotes gemcitabine-induced cytotoxicity and therefore abrogates chemoresistance to gemcitabine. Second, we have confirmed that HMGA1 silencing promotes gemcitabine-induced cytotoxicity and reduces tumor growth in vivo in a nude mouse xenograft model of pancreatic cancer. Finally, our findings suggest a plausible mechanism by which HMGA1 promotes chemoresistance to gemcitabine: activation of Akt signaling. Phosphatidylinositol 3-kinase/Akt signaling is well described as a mediator of chemoresistance to gemcitabine in the context of pancreatic cancer (19, 26, 27). These findings imply that targeted suppression or inactivation of HMGA1 could be a potential therapeutic strategy for increasing chemosensitivity to gemcitabine in this highly chemoresistant cancer.

Fig. 4. We have previously reported that in MiaPaCa2 cells, HMGA1 silencing reduces Akt phosphorylation whereas HMGA1 overexpression promotes Akt phosphorylation, and neither HMGA1 silencing nor overexpression have any effect on the level of expression of total Akt. To assess the role of Akt in mediating the HMGA1-induced chemoresistance, we transduced pIRES-HMGA1 and pIRES-HMGA1.2 clones with adenovirus carrying hemagglutinin-tagged dominant negative Akt to examine its effects on chemoresistance. Transduction efficiency and expression of dominant negative Akt were assessed by Western blotting for hemagglutinin. Infection of pIRES-HMGA1 and pIRES-HMGA1.2 clones with adenovirus carrying dominant negative Akt (Ad-DN-Akt) resulted in significant reductions in IC50 to gemcitabine when compared with cells infected with control adenovirus (Ad-CMV-Null). Dominant negative Akt resulted in reductions in IC50 to gemcitabine in pIRES-HMGA1 and pIRES-HMGA1.2 clones to levels similar to parental MiaPaCa2 cells or empty pIRES-puro3 transfectants, indicating abrogation of the increased chemoresistance associated with HMGA1 overexpression.

Fig. 5. A, stable silencing of HMGA1 promoted chemosensitivity to gemcitabine in vivo with evidence of tumor regression in nude mouse s.c. model. Mice (n = 8 per group) were s.c. implanted with 2 × 106 lentivirus-mediated stable transfectant BxPC3 cells (either shHMGA1 or control shRNA). Gemcitabine treatment was commenced in each group 14 d after implantation when the tumors were ~50 mm3 in volume. Mice received gemcitabine (150 mg/kg) in 100 μL of PBS vehicle by twice-weekly i.p. injection. Subcutaneous tumor size was monitored weekly during the 6 wk of treatment. Tumors with HMGA1 silencing showed evidence of regression in size during the treatment period whereas tumors in the control group continued to grow with time. Points, mean; bars, SD. *, P < 0.05 versus control shRNA xenografts. B, representative photograph of one mouse from each group is shown, with tumors located in their flanks. C, the explanted tumors at the end of the 6-wk treatment period. Of note, one of the mice in the shHMGA1 group had its tumor completely regressed during the 6-wk gemcitabine treatment period.
allowing for a more open chromatin structure that facilitates transcriptional activation (28). Further, HMGA1 is able to bind to AT-rich promoter regions, where it modifies DNA conformation to facilitate binding of other transcriptional factors that promote gene transcription (29). By binding to promoter regions, HMGA1 is also able to form multiprotein complexes, the enhanceosomes, that serve as transcription-activating complexes (29). Given these putative functions, it is not surprising that HMGA1 is involved in the regulation of a large number of target genes. Previous studies have shown that HMGA1 overexpression is associated with increased expression of growth factors/cytokines (e.g., fibroblast growth factors, IFNs α and β, and interleukins 10-14 and 17), growth factor receptors (e.g., fibroblast growth factor receptor, epidermal growth factor receptor, ERBB3, and ERBB4), and multiple integrins (α1, α6, α9, β1, β3, β6; ref. 24). Clearly, it is not surprising that through induction of these growth factor–related signaling pathways, HMGA1 could have an effect on prosurvival phosphatidylinositol 3-kinase/Akt pathways, as shown in this study. It is also plausible that by increasing integrin expression, HMGA1 may stimulate integrin-linked kinase, which is known to directly interact with integrins and phosphorylate Akt in a phosphatidylinositol 3-kinase–dependent manner (30).

In the current study, we have examined the effects of silencing HMGA1 on chemoresistance in BxPC3 and MiaPaCa2 pancreatic adenocarcinoma cells. BxPC3 cells inherently express relatively high levels of HMGA1 whereas MiaPaCa2 cells inherently express relatively low levels of HMGA1 under baseline conditions. Because these cell lines differ in ways other than in HMGA1 expression levels alone, we examined the effects of modulating HMGA1 expression in a single cell line system. This approach allowed us to control for potential confounders, such as variable K-ras mutation status (31), inherent in comparisons involving multiple cell lines. We did both loss-of-function (HMGA1 silencing) and gain-of-function (HMGA1 overexpression) experiments on MiaPaCa2 cells. As such, by using the same cell line and theoretically controlling for variations in genetic background, we were able to show that HMGA1 silencing reduces chemoresistance whereas HMGA1 overexpression results in the reverse effects on chemoresistance.

Although gemcitabine-based regimens are currently standard of care for the treatment of advanced pancreatic cancer, their efficacy is limited by profound chemoresistance. Recently, the combination of gemcitabine and the human epidermal growth factor receptor tyrosine kinase inhibitor erlotinib has been reported, for the first time, to be associated with improved
survival over single-agent gemcitabine in patients with advanced pancreatic cancer in a phase III clinical trial (32). Although these results are encouraging, the benefits provided by the addition of erlotinib are only incremental. Truly transformative increases in efficacy are likely to arise only through incorporation of targeted therapies selected on the basis of rational understanding of mechanisms mediating chemoresistance in pancreatic cancer. In this context, our findings suggest that HMGA1 warrants further investigation as a novel therapeutic target in this deadly cancer.

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