Research Article

Silencing of High Mobility Group Isoform I-C (HMGI-C) Enhances Paclitaxel Chemosensitivity in Breast Adenocarcinoma Cells (MDA-MB-468)

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Abstract

Purpose: HMGI-C (High Mobility Group protein Isoform I-C) protein is a member of the high-mobility group AT-hook (HMGA) family of small non-histone chromosomal protein that can modulate transcription of an ample number of genes. Genome-wide studies revealed up regulation of the HMGI-C gene in many human cancers. We suggested that HMGI-C might play a critical role in the progression and migration of various tumors. However, the exact role of HMGI-C in breast adenocarcinoma has not been cleared.

Methods: The cells were transfected with siRNAs using transfection reagent. Relative HMGI-C mRNA and protein levels were measured by quantitative real-time PCR and Western blotting, respectively. The cytotoxic effects of HMGI-C siRNA, Paclitaxel alone and combination on breast adenocarcinoma cells were determined using MTT assay. The migration after treatment by HMGI-C siRNA, Paclitaxel alone and combination were detected by wound-healing respectively.

Results: HMGI-C siRNA significantly reduced both mRNA and protein expression levels in a 48 hours after transfection and dose dependent manner. We observed that the knockdown of HMGI-C led to the significant reduced cell viability and inhibited cells migration in MDA-MB-468 cells *in vitro*.

Conclusion: These results propose that HMGI-C silencing and Paclitaxel treatment alone can inhibit the proliferation and migration significantly, furthermore, synergic effect of HMGI-C siRNA and Paclitaxel showed higher inhibition compared to mono treatment. Taken together, HMGI-C could be used as a promising therapeutic agent in the treatment of human breast adenocarcinoma. Therefore HMGI-C siRNA may be an effective adjuvant in human breast adenocarcinoma.

Introduction

Breast cancer is the most common cancer diagnosed among women in the worldwide, accounting for nearly 1 in 3 cancers, also it is the second cause of cancer death among women fallowing lung cancer.[1] Despite the great improvements in the clinical and therapeutic techniques in recent years, many advanced breast cancer patients still die of the postoperative recurrence and metastasis of disease. One of the primary reasons for ineffective therapies for these patients is our lack of understanding about the complete and accurate molecular mechanisms involved in carcinogenesis, progression and invasion of breast cancer. Therefore, the innovation of new treatment modalities to overcome these ineffective therapies are necessary.[2] Effectiveness of siRNA in cancer treatment has been specified by: potential and high efficiency, knock downing in the advanced stages of growth and low cost compared to the other methods of gene therapy,[3-5]

and high specificity contrasted to the other cancer therapy methods such as operation and chemotherapy.[6,7]

HMGI-C protein, also known as HMGA2 protein, belongs to the family of nuclear non-histone phosphoproteins called high mobility group A (HMGA). These proteins have a relatively small molecular weight about 12 kDa and contain three basic short sequences, called the AT-hook. These basic sequences bind to AT-rich regions of the minor groove of B-form DNA. HMGA proteins are involved in many fundamental cellular processes, including mitosis, cell-cycle control, cell division, regulation of transcription (by binding transcription factors such as NF-kB, ATF-2/c-Jun, Elf-1, Oct-2, Oct-6, SRF, NF-Y, PU-1, RAR), differentiation and cellular aging.[8,9]

HMGA protein family is relatively over expressed, where cells proliferate rapidly, as in the early embryo. *HMGA1* genes are expressed in the tissues of

parenchymal organs and proliferating epithelial cells,[10] whereas the HMGI-C gene highly expressed in all mesenchymal cell condensations and in mesenchymal derivatives.[11] Expression of *HMGA* genes are suppressed in differentiated cells and the HMGI-C gene is under expressed in adult human tissues, other than embryonic tissues.[12,13]

Over expression of HMGI-C gene was observed in many human malignancies such as non-small lung cancers,[14] pancreatic carcinoma,[15] epithelial ovarian cancers,[16] colorectal cancer,[17] retinoblastomas,[18] squamous cell carcinomas,[19] myeloproliferative disorder[20] and it has also been found to participate in EMT.[21,22]

In this study we investigated whether the down-regulation of HMGI-C level by siRNA could sensitize breast adenocarcinoma cells to Paclitaxel. To this end, we examined the effects of either HMGI-C specific siRNA or Paclitaxel treatments alone versus the combination, on invasion and survival invitro, in MDA-MB-468 cell line.

Materials and Methods
Materials
Human HMGI-C siRNA, goat polyclonal anti-HMGI-C antibody, monoclonal b-actin antibody, siRNA transfection reagent and siRNA transfection medium were purchased from Santacruz biotechnology (California, USA). Rabbit anti-goat antibody was purchased from Cytomatin gene company (Isfahan, Iran), rabbit anti-mouse anti-body was purchased from Razi institute. Paclitaxel was purchased from activis (Milan, Italy).QRT-PCR master mix was purchased from Takara bio Inc. (Shiga, Japan).

Cell culture
The human breast adenocarcinoma cell line, MDA-MB-468 was purchased from Pasture institute (Tehran, Iran). The MDA-MB-468 breast cells were maintained in RPMI-1640 culture medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), antibiotics (100 IU/ml penicillin, 100 µg/ml streptomycin) (Gibco, USA) at 37 °C in a 95% humidified atmosphere containing 5% CO2. Cells were grown on sterilized culture dishes and were passaged every 3 days following 0.25 % trypsin/EDTA (Gibco, USA) digestion.

siRNA transfection
MDA-MB-468 cells were cultured at a density of 2×10^5 cells/ml of six-well plates and transfected at 60-80% confluency. siRNA transfection (at a final concentration of 80 pmol in all experiments) was performed using siRNA transfection reagent (Santacruz biotechnology, USA) according to the manufacturer's recommendations. Briefly, siRNA and siRNA transfection reagent were diluted in siRNA transfection medium (Santacruz biotechnology, USA) separately. The diluted solutions were then mixed and incubated for 15-30 min at room temperature. Subsequently, the mixtures were added to each well containing cells and transfection medium.

After 5-7 hr transfection, RPMI medium containing final FBS concentration of 20% was added into transfected wells. After 48 hr of incubation, down-regulation of HMGI-C was measured using qRT-PCR. Then, Western blot was utilized to test the target protein to ensure the transfection efficiency. The suppression of HMGI-C expression was then assessed by quantitative real-time PCR (qRT-PCR) and Western blotting.

Real-time quantitative PCR
Total- RNA was extracted using AccuZolTM reagent (Bioneer, Daedeok-gu, Daejeon, Korea) as described by the manufacturer's protocol. The mRNA was reverse-transcribed into cDNA from 1 µg of total RNA by use of MMLV reverse transcriptase (Promega, Madison, WI, USA) and oligo-dT primer according to the manufacturer's instructions. The expression level of HMGI-C mRNA was carried out by qRT-PCR using SYBR Premix Ex Taq (Takara Bio, Otsu, Shiga, Japan) and Rotor- GeneTM 6000 system (Corbett Life Science, Mortlake, NSW, Australia). The reaction system of PCR was: 12 µl of SYBR green reagent, 0.2 µM of each primer, 1 µl of cDNA template, and 6 µl of nuclease-free distilled water. All pair primer sequences were blasted using the primer-blast software on the NCBI website (http://www.nchi.nlm.nih.gov) prior to the experiment. The primer sequences were as follows: forward, 5'-TGGGAGGAGCGAAATCTAAA-3', reverse, 5'-TGGTATTCAGGTCTTTCATGG-3', for HMGI-C, and forward, 5'-TCCCTGGAGAAGAGCTACG-3', and reverse, 5'-GTAGTTTCGTGGATGCCACA-3', for β–actin. The initial denaturation step at 95°C for 10 min was followed by 45 cycles at 95°C for 10 sec, 59°C for 30 sec and 72°C for 20 sec. β-actin was used as the reference gene. The relative levels of gene expression were calculated by the $2^{-\Delta\Delta Ct}$ method. All qRT-PCR reactions were performed in triplicate.

Western blot analysis
Briefly, total protein from cells was extracted using RIPA buffer (25 mM Tris HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing protease inhibitor cocktail (Roche Diagnostics GmbH). Suspensions were centrifuged at 14,000 rpm for 10 min at 4°C and cellular debris was discarded. Protein concentrations were quantified using NanoDrop (Thermo scientific, Wilmington, USA). Fifty micrograms of each protein sample were mixed with protein sample buffer (10% Glycerol, 50 Mm Tris pH 8.6, 2% SDS, 1% Bromophenol blue, and 100 mM DTT) and the samples were incubated for 5 min in a boiling water bath, then separated on 12.5% SDS-polyacrylamide gel electrophoresis. After transferring separated proteins to an activated polyvinylidine diflouride (PVDF) membranes, we blocked the membranes with 0.5% tween 20 in PBS/Tween-20 (0.05%, v/v) for 24 hr at 4°C. Following on, the membranes were probed 1 hr at room temperature with primary goat poly clonal antibodies against HMGI-C (1:2000, Santacruze biotechnology,

California, USA) and β-actin (1:5000, Santacruze biotechnology, California, USA) diluted in 3% BSA in PBS. After four washes with a buffer containing PBS and 0.05% Tween-20, membranes were incubated with appropriate horseradish peroxidase-linked Rabbit anti-goat secondary antibody (1:5,000, cytomatin gene, Isfahan, Iran) and rabbit anti-mouse antibody (Razi institute, Tehran, Iran) diluted in PBS and 0.05% Tween-20 for 1 hr at room temperature. Subsequently, the membranes were washed and protein bands visualized using enhanced BM chemiluminescence blotting substrate POD (Roche Diagnostics GmbH, Mannheim, Germany) and autoradiography films (Estman Kodak, Rochester, NY, USA). Signals were measured using NIH ImageJ 1.63 Software.

Cytotoxicity assay

Cytotoxicity of the treatments was measured using a methylthiazol tetrazolium (MTT) assay kit (Sigma). The experiment was subdivided into eight groups: 80 pmol HMGI-C siRNA, Paclitaxel in 6 different doses around IC50 and combination of 80 pmol HMGI-C siRNA with 6 different paclitaxel doses. Briefly, cells were cultured at a density of 15×10^3 cells/well in 96-well cell culture plates and then transfected with siRNAs. After 48 hr of incubation, the cells were exposed to different concentrations of paclitaxel (0.39, 0.78, 1.56, 3.25, 6.5 and 12.5 μM). After a total 24 hr of treatment, 50μl of MTT (2mg/ml in PBS) was added to each well and then incubated for a further 4 hr. The formazan crystals were formed by adding 200 μl of the solubilization (DMSO + Sorensen buffer) to each well. After 30 minutes incubation in above-mentioned conditions, the optical density (OD) of each well was measured at a wavelength of 570nm using an ELISA reader (Awareness Technology, Palm City, FL, USA) and all experiments were performed in triplicate.

Combination effect analysis

The combination effect between HMGI-C siRNA and paclitaxel were evaluated, based on the principles described by Chou and Talalay.[23] For each combination experiment, a coefficient of drug interaction (CDI) number was calculated using the following formula: CDI=SAB /(SA×SB), were introduced SA as paclitaxel, SB as HMGI-C siRNA and SAB as combination treatment survival rate, relative to the control. Combination effects were assessed after 48 hr of treatment. This method of analysis generally defines CDI<1 as synergistic, CDI=1 as additive and CDI>1 as antagonistic effects, respectively.

Migration assay

MDA-MB-468 cells migration was measured by using a wound-healing assay (Scratch). MDA-MB-468 cells (4×10^5 cells/well) were placed for 24 h in 6-well plates and a wound was made by using yellow pipette tip across the cell monolayer to create an open gap, mimicking a wound when the cultured cells reached >90 %

confluence and cell debris were removed by washing with serum-free medium. Then cells on the plate were photographed under the light microscope (time 0) and then incubated for another 48 hr at 37 °C 5% CO2 and allowed to migrate into the wound area. Images of the wound were collected at 0 and 48 hr using a microscope. The migration rate was quantified by measuring the distance between the wound edges. This assay was independently repeated for three times.

Statistical analysis

All results in this study were presented as mean ± standard deviation (SD). Statistical significance of differences between groups was explored by using student T test and One-way ANOVA followed by Dunnett's multiple comparisons using GraphPad Prism software, La Jolla California USA, http://www.graphpad.com. Value of P less than 0.05 was considered significant.

Results

### siRNA suppressed HMGI-C mRNA and protein levels in breast adenocarcinoma cells

First, we explored the effect of siRNA on HMGI-C gene expression in MDA-MB468 cells by qRT-PCR and Western blot analysis. Relative HMGI-C gene expression was calculated in relation to the control group, which was considered as 100%. As shown in Figure 1A,1B, 2A and 2B, HMGI-C siRNA led to a marked reduction of HMGI-C mRNA in both dose – dependent mRNA and protein levels (p<0.05; relative to the control). At 24, 48 and 72hr after the transfection, the relative HMGI-C mRNA expression levels were 77.06%, 54.10% and 78.95%, respectively (Figure 1A), and the dose-dependent at 40, 60, 80 pmol of HMGI-C siRNA transfection relative HMGI-C mRNA expression levels were 88.87%, 71.13% and 47.25% (Figure 1B), and HMGI-C protein expression levels were 67.10%, 11.65% and 8.20%, respectively (Figure 2A, 2B) (p<0.05).

### HMGI-C siRNA synergistically enhanced the cytotoxic effect of paclitaxel

To assess whether down-regulation of HMGI-C could enhance the sensitivity of the breast adenocarcinoma cells to paclitaxel, HMGI-C siRNA and Paclitaxel alone and combination treatment of paclitaxel and HMGI-C siRNA were investigated on MDA-MB468 cells (Figure 3A, 3B). As shown in Figure 3B, mono treatment with paclitaxel induced cytotoxicity in a dose-dependent way. The results of MTT assay showed that HMGI-C siRNA significantly decreased the cell survival rate to 43.24%, compared with the control group (p<0.05). Moreover, paclitaxel in combination with HMGI-C siRNA further decreased the cell survival rate relative to paclitaxel or HMGI-C siRNA alone (p<0.05). The CDI values were also less than 1 in all concentrations of Paclitaxel, which indicated the synergistic effect between the two agents (Figure 3B).
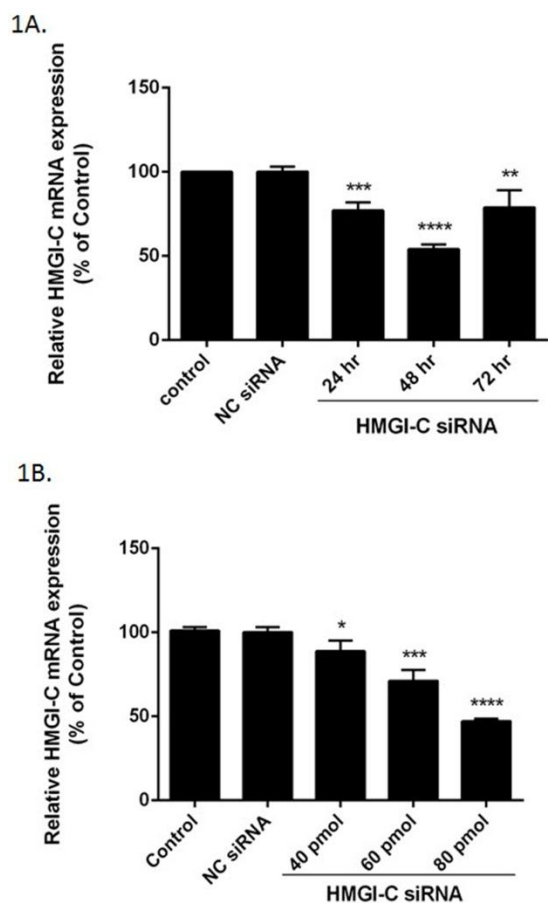
Figure 1. Suppression of HMGI-C mRNA Expression by siRNA in breast adenocarcinoma. (A) MDA-MB-468 cells were transfected with HMGI-C siRNA for 24, 48 and 72 hr. **(B)** MDA-MB-468 cells were transfected with HMGI-C siRNA with the doses of 40, 60, 80 pmol. Relative HMGI-C mRNA expression was measured by qRT-PCR using $2^{(-\Delta\Delta Ct)}$ method. The results are expressed as mean±SD (n=3); *p<0.05, **p<0.001, *** p= 0.0001 and ****p<0.0001 versus control.
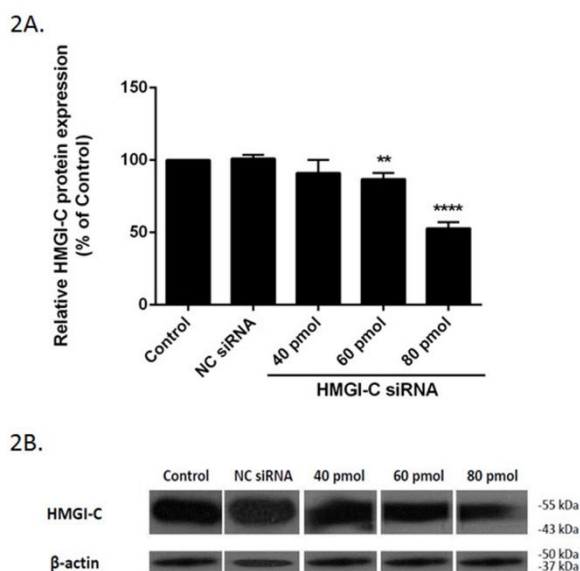

Figure 2. HMGI-C Protein Expression in MDA-MB-468 cells Transfected with siRNA. **(A)** Representative western blot of Beta-actin and HMGI-C proteins from cells transfected with HMGI-C siRNA. **(B)** The expression level of each band was quantified using densitometry and normalized to the respective Beta-actin. The results are expressed as mean±SD (n=3); **p<0.001 and ****p<0.0001, versus control.
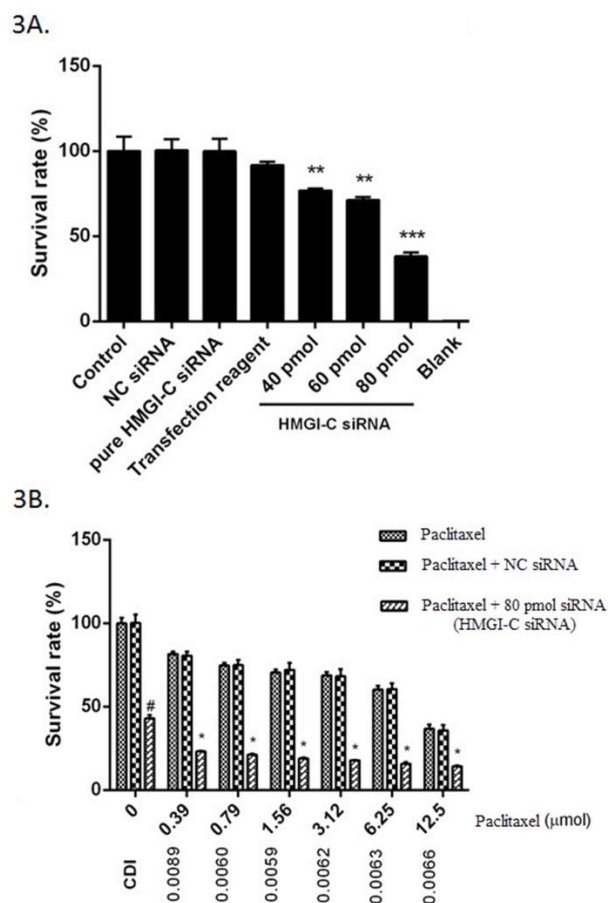
Figure 3. Effect of HMGI-C siRNA on the Chemosensitivity of the MDA-MB-468 Cells. (A) 48 hr after transfection with HMGI-C siRNA (40, 60, 80 pmol), cytotoxicity of treatments was determined by MTT assay, **P<0.01 and ***P<0.001, versus control. **(B)** The cells were treated with Paclitaxel, and combination of paclitaxel and 80 pmol HMGI-C siRNA for 48 hr and then the cytotoxicity of the treatments was determined by MTT assay. The interaction effect between siRNA and Paclitaxel was determined using combination effect analysis and coefficient of drug interaction (CDI) values. The results are expressed as mean±SD (n=3); #p<0.05 versus 80pmol HMGI-C siRNA alone; *p<0.0001 versus control group.

Suppression of HMGI-C inhibited cells migration

Down-regulation of HMGI-C by using siRNA blocks migration of breast adenocarcinoma cells in vitro. Thus, we sought to determine whether siRNA blocks migration and invasion of breast cancer cells in vitro. Wound healing assays were performed to examine whether migration of the MDA-MB-468 cells transfected with HMGI-C siRNA was inhibited. Results showed that the knockdown of HMGI-C with siRNA, or Paclitaxel treatments alone versus the combination blocked the migration of MDA-MB-468 cells (Figure 4B). The number of MDAMB-468 cells with HMGI-C siRNA in 48 hr after treatment was 38.30%, Paclitaxel was 34.57%, and combination of siRNA and Paclitaxel was 24.61% that migrated to the scratched area of that of control (untreated) MDA-MB-468 cells (Figure 4A, 4B) (P < 0.0001). The CDI values were also less than 1 indicated the synergistic effect between the two agents (Figure 4B).

4A.

4B.
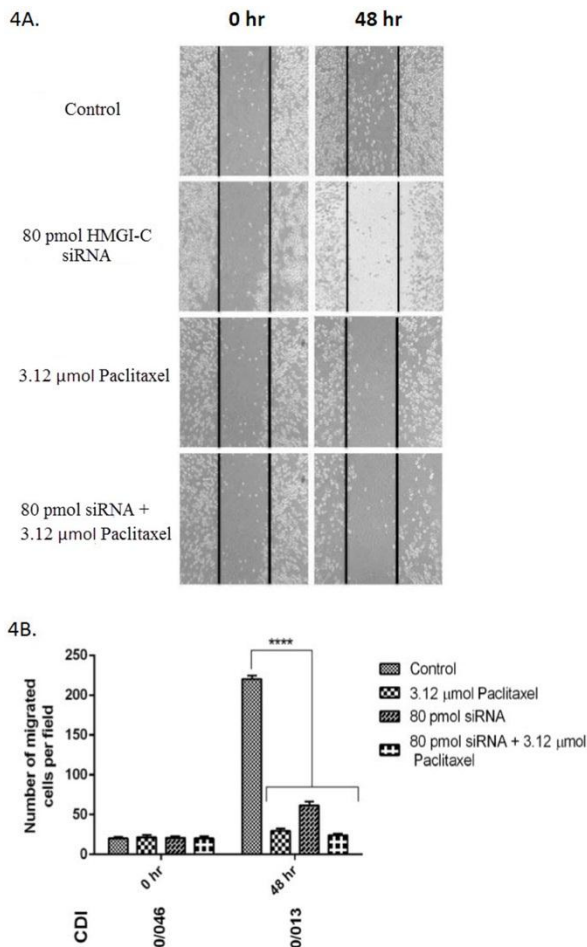
Figure 4. Untreated and treated MDA-MB-468 cells were subjected to scratch wound-healing assays. (A) MDA-MB-468 cells divided in 4 groups including untreated, specific siRNA, Paclitaxel and combination specific siRNA and Paclitaxel. The wound space was photographed at 0 and 48 hr. (B) The number of migrated cells to the wound sides was evaluated and statistically analyzed. Error bars indicate the mean ± SD of three independent experiments (****$P < 0.0001$).

Discussion

Previous studies showed that HMGI-C increases tumor transformation in different cell types.[24,25] It has been found that HMGI-C overexpression is associated with enhancing tumor growth and invasion, early metastasis, and a poor prognosis, typically seen in pancreatic cancer,[26] papillary thyroid carcinoma,[27] colorectal cancer,[17] ovarian cancer,[28] lung cancer[14] and HG-PSC.[16] Previous studies have demonstrated that, HMGI-C gene suppression leads to increased apoptosis and simultaneously sensitizes the malignant cells to chemotherapeutic agents. In this regards, we utilized RNA interference for specific suppression of HMGI-C in MDA-MB-468 cells to overcome resistance to chemotherapeutic agent, paclitaxel. In the current study we examined the effect of HMGI-C specific siRNA and paclitaxel alone or in combination on MDA-MB-468 breast adenocarcinoma cells cytotoxicity and migration.

The results of qRT-PCR and western blot analysis showed that transfection with HMGI-C siRNA could significantly reduce the mRNA levels of HMGI-C and its translated protein during the 48 hr period in dose dependent manner, suggesting that HMGI-C siRNA could effectively cleave HMGI-C mRNA and blocked its translation to protein.

As our previous study treatment of adenocarcinoma cells with specific HMGI-C siRNA induced apoptosis and cell cycle arrest. The results of MTT assay revealed that pretreatment with HMGI-C siRNA could synergistically reduce the viability of breast malignant cells to paclitaxel, demonstrating that HMGI-C down-regulation could sensitize MDA-MB-468 cells to paclitaxel. we examined the rate of migration through wound-healing (scratch) assay, It was found that migration were significantly inhibited in MDA-MB-468 cell lines after HMGI-C siRNA and paclitaxel alone or in combination treatment compare to control (untreated) group.

In this study, we found that HMGI-C siRNA enhances paclitaxel chemosensitivity and inhibits migration in breast adenocarcinoma cells. Cell cytotoxicity and migration of HMGI-C siRNA transfected, and paclitaxel treated cell lines notably were declined compared to those of untreated cells and the cells that treated with HMGI-C siRNA and paclitaxel individually.

CDI value showed that treatment with HMGI-C siRNA and paclitaxel inhibit cells migration in a synergic manner compared to HMGI-C siRNA group, paclitaxel group and control group (untreated). In similar study, Karami et al. showed specific silencing of survivin expression by siRNA enhanced sensitivity of leukemic cells to etoposide.[29,30] In other studies, they showed MDR1 down-regulation synergistically increased the cytotoxic effects of oxaliplatin and etoposide on oxaliplatin resistant SW480 and etoposide resistant HL-60 cells.[5,31]

In summary, we demonstrated for the first time to our knowledge that, HMGI-C overexpression was sufficient to induce tumor formation and cell migration in MDA-MB-468 cell lines. We postulate that induction of HMGI-C up regulation in early breast adenocarcinoma may be responsible for rapid progression of breast adenocarcinoma. Further characterization of the functional relationship between HMGI-C and HMGI-C-mediated migration target gene regulation will help us understand the tumor genesis of breast adenocarcinoma.

Conclusion

Our data suggest that specific HMGI-C siRNA can inhibit the expression of HMGI-C protein and mRNA in breast adenocarcinoma cells and may potentially be a therapeutic agent for breast cancer metastasis. It can also synergetic effect with paclitaxel and decrease the effective dose of paclitaxel in treatment of breast adenocarcinoma cells.

Acknowledgments

Ethical Issues
Not applicable.

Conflict of Interest
Authors declare no conflict of interest in this study.

Abbreviations
siRNA, small interfering RNA; HMGI-C, High mobility group protein isoform I-C; HMGA1, high mobility group A1; HMGA2, high mobility group A1; EMT, *Epithelial-mesenchymal transition*; MDA-MB-468, Breast adenocarcinoma cell line; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; ATF2, Activating transcription factor 2; ELF1, E74-like factor 1; OCT2, Octamer transcription factor 2; Oct-6, Octamer transcription factor 6; *SRF, Serum response factor;* NF-Y, Nuclear factor Y; RAR, *Retinoic acid receptor*

References
1. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. *CA Cancer J Clin* 2014;64(1):52-62. doi: 10.3322/caac.21203
2. Whelan J. First clinical data on rnai. *Drug Discov Today* 2005;10(15):1014-5. doi: 10.1016/S1359-6446(05)03547-6
3. Fire AZ. Gene silencing by double-stranded rna. *Cell Death Differ* 2007;14(12):1998-2012. doi: 10.1038/sj.cdd.4402253
4. Yazdi Samadi BVM. Genetics a molecular approach. Iran;Tehran University Publishers: Iran: Tehran University Publishers; 1388.
5. Kachalaki S, Baradaran B, Majidi J, Yousefi M, Shanehbandi D, Mohammadinejad S, et al. Reversal of chemoresistance with small interference rna (sirna) in etoposide resistant acute myeloid leukemia cells (hl-60). *Biomed Pharmacother* 2015;75:100-4. doi: 10.1016/j.biopha.2015.08.032
6. Mansoori B, Sandoghchian Shotorbani S, Baradaran B. Rna interference and its role in cancer therapy. *Adv Pharm Bull* 2014;4(4):313-21. doi: 10.5681/apb.2014.046
7. Mansoori B, Mohammadi A, Shir Jang S, Baradaran B. Mechanisms of immune system activation in mammalians by small interfering rna (sirna). *Artif Cells Nanomed Biotechnol* 2015:1-8. doi: 10.3109/21691401.2015.1102738
8. Narita M, Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, et al. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. *Cell* 2006;126(3):503-14. doi: 10.1016/j.cell.2006.05.052
9. Reeves R. Molecular biology of hmga proteins: Hubs of nuclear function. *Gene* 2001;277(1-2):63-81.
10. Chiappetta G, Avantaggiato V, Visconti R, Fedele M, Battista S, Trapasso F, et al. High level expression of the hmgi (y) gene during embryonic development. *Oncogene* 1996;13(11):2439-46.
11. Hirning-Folz U, Wilda M, Rippe V, Bullerdiek J, Hameister H. The expression pattern of the hmgic gene during development. *Genes Chromosomes Cancer* 1998;23(4):350-7.
12. Gattas GJ, Quade BJ, Nowak RA, Morton CC. Hmgic expression in human adult and fetal tissues and in uterine leiomyomata. *Genes Chromosomes Cancer* 1999;25(4):316-22.
13. Rogalla P, Drechsler K, Frey G, Hennig Y, Helmke B, Bonk U, et al. Hmgi-c expression patterns in human tissues. Implications for the genesis of frequent mesenchymal tumors. *Am J Pathol* 1996;149(3):775-9.
14. Meyer B, Loeschke S, Schultze A, Weigel T, Sandkamp M, Goldmann T, et al. Hmga2 overexpression in non-small cell lung cancer. *Mol Carcinog* 2007;46(7):503-11. doi: 10.1002/mc.20235
15. Abe N, Watanabe T, Suzuki Y, Matsumoto N, Masaki T, Mori T, et al. An increased high-mobility group a2 expression level is associated with malignant phenotype in pancreatic exocrine tissue. *Br J Cancer* 2003;89(11):2104-9. doi: 10.1038/sj.bjc.6601391
16. Malek A, Bakhidze E, Noske A, Sers C, Aigner A, Schafer R, et al. Hmga2 gene is a promising target for ovarian cancer silencing therapy. *Int J Cancer* 2008;123(2):348-56. doi: 10.1002/ijc.23491
17. Guang-meng XU, Hai-na Z, Xiao-feng T, Mei S, Xue-dong F. Effect of HMGA2 shRNA on the cell proliferation and invasion of human colorectal cancer sw480 cells in vitro. *Chem Res Chinese Univ* 2012;28(2):264-8.
18. Chau KY, Manfioletti G, Cheung-Chau KW, Fusco A, Dhomen N, Sowden JC, et al. Derepression of hmga2 gene expression in retinoblastoma is associated with cell proliferation. *Mol Med* 2003;9(5-8):154-65.
19. Miyazawa J, Mitoro A, Kawashiri S, Chada KK, Imai K. Expression of mesenchyme-specific gene hmga2 in squamous cell carcinomas of the oral cavity. *Cancer Res* 2004;64(6):2024-9.
20. Andrieux J, Demory JL, Dupriez B, Quief S, Plantier I, Roumier C, et al. Dysregulation and overexpression of hmga2 in myelofibrosis with myeloid metaplasia. *Genes Chromosomes Cancer* 2004;39(1):82-7. doi: 10.1002/gcc.10297
21. Lee YS, Dutta A. The tumor suppressor microrna let-7 represses the hmga2 oncogene. *Genes Dev* 2007;21(9):1025-30. doi: 10.1101/gad.1540407
22. Thuault S, Tan EJ, Peinado H, Cano A, Heldin CH, Moustakas A. Hmga2 and smads co-regulate snail1 expression during induction of epithelial-to-mesenchymal transition. *J Biol Chem* 2008;283(48):33437-46. doi: 10.1074/jbc.M802016200
23. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 1984;22:27-55.
24. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and hmga2 enhances oncogenic

transformation. *Science* 2007;315(5818):1576-9. doi: 10.1126/science.1137999

25. Di Cello F, Hillion J, Hristov A, Wood LJ, Mukherjee M, Schuldenfrei A, et al. Hmga2 participates in transformation in human lung cancer. *Mol Cancer Res* 2008;6(5):743-50. doi: 10.1158/1541-7786.MCR-07-0095

26. Hristov AC, Cope L, Reyes MD, Singh M, Iacobuzio-Donahue C, Maitra A, et al. Hmga2 protein expression correlates with lymph node metastasis and increased tumor grade in pancreatic ductal adenocarcinoma. *Mod Pathol* 2009;22(1):43-9. doi: 10.1038/modpathol.2008.140

27. Chiappetta G, Ferraro A, Vuttariello E, Monaco M, Galdiero F, De Simone V, et al. Hmga2 mrna expression correlates with the malignant phenotype in human thyroid neoplasias. *Eur J Cancer* 2008;44(7):1015-21. doi: 10.1016/j.ejca.2008.02.039

28. Wu J, Liu Z, Shao C, Gong Y, Hernando E, Lee P, et al. Hmga2 overexpression-induced ovarian surface epithelial transformation is mediated through regulation of emt genes. *Cancer Res* 2011;71(2):349-59. doi: 10.1158/0008-5472.CAN-10-2550

29. Karami H, Baradaran B, Esfahani A, Estiar MA, Naghavi-Behzad M, Sakhinia M, et al. Sirna-mediated silencing of survivin inhibits proliferation and enhances etoposide chemosensitivity in acute myeloid leukemia cells. *Asian Pac J Cancer Prev* 2013;14(12):7719-24.

30. Karami H, Baradaran B, Esfehani A, Sakhinia M, Sakhinia E. Down-regulation of mcl-1 by small interference rna induces apoptosis and sensitizes hl-60 leukemia cells to etoposide. *Asian Pac J Cancer Prev* 2014;15(2):629-35.

31. Montazami N, Kheir Andish M, Majidi J, Yousefi M, Yousefi B, Mohamadnejad L, et al. Sirna-mediated silencing of mdr1 reverses the resistance to oxaliplatin in sw480/oxr colon cancer cells. *Cell Mol Biol (Noisy-le-grand)* 2015;61(2):98-103.