Regulation of Minichromosome Maintenance Gene Family by MicroRNA-1296 and Genistein in Prostate Cancer

Shahana Majid¹, Altaf A. Dar², Sharanjot Saini¹, Yi Chen¹, Varahram Shahryari¹, Jan Liu¹, Mohd Saif Zaman¹, Hiroshi Hirata¹, Soichiro Yamamura¹, Koji Ueno¹, Yuichiro Tanaka¹, and Rajvir Dahiya¹

Abstract

The minichromosome maintenance (MCM) gene family is essential for DNA replication and is frequently upregulated in various cancers. Here, we examined the role of MCM2 in prostate cancer and the effect of microRNA-1296 (miR-1296), genistein, and trichostatin A (TSA) on the MCM complex. Profiling results showed that expression of MCM genes was higher in tumor samples. Genistein and TSA significantly downregulated the expression of all MCM genes. Genistein, TSA, and small interfering RNA duplexes caused a significant decrease in the S phase of the cell cycle. There was also downregulation of CDT1, CDC7, and CDK2 genes, which govern loading of the MCM complex on chromatin. We also found that miR-1296 was significantly downregulated in prostate cancer samples. In PC3 cells, inhibition of miR-1296 upregulated both MCM2 mRNA and protein, whereas overexpression caused a significant decrease in MCM2 mRNA, protein, and the S phase of the cell cycle. MCM genes are excellent anticancer drug targets because they are essential DNA replication factors that are highly expressed in cancer cells. This is the first report showing anti-MCM effect by miR-1296, genistein, and TSA. TSA is undergoing clinical trials as a prostate cancer treatment but has high toxicity. Genistein, a natural, nontoxic dietary isoflavone, may be an advantageous therapeutic agent for treating prostate cancer. The use of RNA interference is currently being implemented as a gene-specific approach for molecular medicine. The specific downregulation of oncogenes by miR may contribute to novel therapeutic approaches in the treatment of prostate cancer. Cancer Res; 70(7); 2809–18. ©2010 AACR.

Introduction

DNA replication occurs in a precise fashion during eukaryotic cell division. This tight control is orchestrated by many regulatory molecules, including members of the minichromosome maintenance (MCM) gene family. Initially, MCM proteins are recruited to sites of DNA replication and interact with each other, forming the MCM2-MCM7 complex during the G1 phase of the cell cycle (1). This complex has helicase activity and facilitates DNA replication (2). Therefore, the MCM proteins are essential for proliferating cells and are frequently upregulated in a variety of dysplastic and cancer cells (3, 4). Central to the DNA replication licensing system is the formation of pre-replicative complexes (pre-RC) in late M and early G1 phases and their subsequent activation at the G1-S boundary. Assembly of pre-RCs requires loading of DNA helicase and the MCM2-MCM7 complex onto chromatin in an ORC1-6–dependend, CDC6-dependend, and CDT1-dependend manner (licensing refs. 5, 6). At the G1-S transition, the activity of two kinases, CDC7 and cyclin E/A-CDK2, recruits additional factors to pre-RCs, resulting in the formation of pre-licensing complexes (pre-Icc; refs. 7, 8). In addition, CDC7 and CDK2 activate the putative MCM2-MCM7 helicase, which, together with pre-Ic formation, results in recruitment of DNA polymerases and initiation of DNA replication. The importance of the replication licensing system is highlighted by the prevalence of genomic instability resulting from hyperactivation of pre-RCs during the neoplastic process and impaired cell growth associated with failure to assemble pre-RCs (9).

The MCM2-MCM7 helicase is thought to exist as a hexamer containing individual MCM polypeptides (MCM2/3/4/5/6/7). MCM2-MCM7 subunits are evolutionarily conserved and essential genes, with each member possessing conserved Walker A, Walker B, and arginine finger motifs required for ATP binding and hydrolysis. The MCM complex is implicated both in the unwinding of origins of DNA replication during initiation and in replication fork progression (10). In addition to the hexamer, MCM subcomplexes (MCM7/4/6, MCM7/4/6/2, and MCM3/5) have also been reported (9, 11). Within these subcomplexes, MCM7/4/6 is thought to possess core ATPase and DNA helicase activity, whereas MCM5/3/2 provides regulatory roles (12). Furthermore, pairwise ATPases studies in vitro have revealed that MCM3/7, MCM4/7, and MCM2/6 pairs are able to catalyze ATP hydrolysis (13). As a hexamer, the ATPase activity of MCM2-MCM7 is not

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required for loading of the complex onto chromatin, whereas ATP hydrolysis by the hexameric complex is essential for initiation of DNA replication (14).

Because cancer cells are highly proliferative, whereas differentiated somatic cells are usually withdrawn from the cell cycle, cell proliferation markers are effective in cancer diagnosis (15). MCM proteins are good proliferation markers and are expressed at high levels in proliferating cells but not in quiescent somatic cells (16). The cellular levels of these proteins remain constant throughout the cell cycle of proliferating cells (17–20), allowing identification of cancer cells in all stages of the cell cycle. Most importantly, because replication licensing is essential for a quiescent cell to reenter the cell cycle, the MCM proteins are expressed as G0 cells enter the G1 phase even before they engage in active DNA synthesis (21). Therefore, the MCM proteins can be used to identify precancerous cells before malignant transformation is completed, providing more sensitive markers for early cancer diagnosis. A recent study showed that both MCM2 and Ki67 are expressed at high levels in the high growth fraction of B-cell lymphomas (22). In contrast, only MCM2, but not Ki67, is expressed at high levels in the low growth fraction of B-cell lymphomas, indicating that DNA replication licensing occurs in premalignant cells before active proliferation (22). In the last few years, several MCM proteins, including MCM5 (23), MCM2 (24–28), and MCM7 (29, 30), are effective molecular markers for proliferating malignant cells in tumors and precancerous cells from premalignant lesions from the lung (26, 27), kidney (31), and prostate (32). Furthermore, increased expression of MCM2 in prostate (32) and lung cancer cells (26, 27) has been shown to correlate with poorer patient survival rates, suggesting that the MCM proteins are sensitive and versatile diagnostic markers for early cancer detection and promising prognostic markers for monitoring responsiveness of various cancers to treatment regimens.

A critical step in anticancer drug development is the identification of drug targets that are cancer cell specific, essential for proliferation, and with activities suitable for high-throughput screens. The MCM complex proteins are a group of promising candidates that meet all these criteria. The coupling of expression of the MCM proteins with proliferation offers the potential of identifying drugs with low toxicity. Their essential roles in proliferation make them effective targets for drugs to kill cancer cells with high potency. Because cell proliferation is a highly conserved cellular process, anti-MCM molecules may be effective against a broad spectrum of different cancers regardless of their tissue or organ of origin. Although anti-MCM small molecules are most likely to prevent proliferation of cancer cells by blocking replication licensing or DNA synthesis during S phase, a recent observation suggests that they may also kill cancer by inducing cancer cell–specific apoptosis (33, 34).

There are no reports on the effect of genistein, a natural, nontoxic dietary isoflavone, or trichostatin A (TSA), a potent anticancer drug, on the MCM gene family. In this study, we show that both genistein and TSA possess an anti-MCM effect by causing a significant decrease in MCM2 gene expression in prostate cancer. We undertook a RNA interference (RNAi) approach to target the MCM2 gene and studied its functional effects in PC3 and LNCaP prostate cancer cells, comparing the results with genistein and TSA. We also showed that microRNA-1296 (miR-1296) regulates MCM2 expression.

**Materials and Methods**

**Tissue samples and cell culture.** Tissue samples from radical prostatectomy were obtained from the Veterans Affairs Medical Center (San Francisco, CA). Informed consent was obtained from all patients. A board-certified pathologist processed the specimens and samples were snap frozen in liquid nitrogen and stored at −80°C. Human prostate carcinoma cell lines (LNCaP and PC3) were obtained from the American Type Culture Collection. The prostate cancer cell lines were cultured as monolayers in RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone), 50 μg/mL penicillin, and 50 μg/mL streptomycin (Invitrogen) and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37°C. Subconfluent cells (60–70% confluent) were treated with varying concentrations of genistein (0, 25, and 50 μmol/L; Sigma) dissolved in DMSO for 96 h or TSA (100 ng/mL; Sigma) for 24 h, and cells treated with vehicle (DMSO) served as control.

**RNA extraction from clinical samples and cell lines.** Total RNA was extracted using a combination of Trizol reagent (Invitrogen) and RNeasy columns (Qiagen). Fresh prostate tissues, however, were homogenized in 1 mL Trizol reagent. After the addition of 0.2 mL chloroform, samples were centrifuged for 15 min at 14,000 rpm. The aqueous phase was moved to a new centrifuge tube and resuspended with one-half volume of 100% ethanol. Samples were then applied to an RNeasy Mini column. For DNA digestion, Ambion DNA-Free kit was used according to the manufacturer’s protocol. RNA quality was assessed using a NanoDrop ND-1000 (NanoDrop Technologies) spectrophotometer. Extracted RNA was stored at −80°C. Total RNA was extracted from 80% confluent plates of cultured cells using a miRNA easy kit (Qiagen) according to the manufacturer’s directions.

**Quantitative real-time PCR.** Mature miRs and other miRNAs were assayed using Taqman MicroRNA Assays and Gene Expression Assays, respectively, in accordance with the manufacturer’s instructions (Applied Biosystems). All reverse transcription (RT) reactions, including no-template controls and RT minus controls, were run in a 7500 Fast Real Time PCR System (Applied Biosystems). Samples were normalized to RNU48 for miR expression or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for gene expression normalization (Applied Biosystems). Gene expression levels were quantified using the 7500 Fast Real Time Sequence Detection System software (Applied Biosystems). Comparative real-time PCR was done in triplicate, including no-template controls. Relative expression was calculated using the comparative Cq method.

**Immunohistochemistry.** Immunostaining was done on tissue microarray slides containing human normal adjacent...
prostate tissue samples (n = 15), malignant tumor (n = 72), and metastatic carcinomas (n = 8). The slides were deparaffinized and antigen retrieval was carried out by microwaving the slides in 10 mmol/L sodium citrate buffer. Slides were incubated overnight with anti-MCM2 antibody (Cell Signaling). Staining was done using the ImmunoCruz Staining System (Santa Cruz Biotechnology) as per the manufacturer’s instructions.

**Immunoblotting.** Protein was isolated from 70% to 80% confluent plates of cultured cells using the M-PER Mammalian Protein Extraction Reagent (Pierce Biotechnology) following the manufacturer’s directions. Protein concentrations were

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*Figure 1.* The MCM gene family is overexpressed in human prostate cancer. A, relative mRNA expression levels in matched normal (N 1-6) and tumor (T 1-6) samples assessed by qRT-PCR. MCM members are significantly overexpressed in tumor samples compared with matched normal samples. *, statistically significant at $P \leq 0.05$. B, 1 to 4, representative pictures of MCM2 immunohistochemical staining at distinct disease stages. 1, normal; 2, BPH; 3, primary cancer; 4, metastatic. In tumor samples, staining was uniformly confined to the nuclear compartment, with rare cytoplasmic staining. C, summarized quick score (quick score = percent cells stained × intensity of stain) results from immunohistochemistry of MCM2 in prostatic tissue arrays.
determined by the Bradford method. Equal amounts of protein were resolved on 10% or 15% SDS-polyacrylamide gels and transferred to nitrocellulose membrane by voltage gradient transfer. The resulting blots were blocked with 5% nonfat dry milk and probed with antibodies specific for MCM2 (Cell Signaling), MCM3 (Cell Signaling), MCM4 (Abcam), MCM5 (Abcam), MCM6 (Abcam), MCM7 (Cell Signaling), CDT1 (Cell Signaling), CDK2 (Cell Signaling), CDC7 (Cell Signaling), and GAPDH (Cell Signaling). Blots were then incubated with appropriate peroxidase-conjugated secondary antibodies and visualized using enhanced chemiluminescence (Pierce Biotechnology).

MCM2 knockdown using small interfering RNA. Prostate cancer cells were plated 24 h before transfection. At 30% to 50% confluence, cells were transfected using Lipofectamine 2000 (Invitrogen) with small interfering RNA (siRNA) duplexes specific for human MCM2 (Qiagen) or control nonsilencing siRNA for 72 h. Initially, three different sets of siRNA duplexes were tested to evaluate the target specificity and knockdown efficiency. Two siRNA duplexes showing the greatest knockdown effect were used for further experiments at 50 nmol/L concentration.

Cell cycle analysis. Cells were harvested, washed with cold PBS, and resuspended in 4′,6-diamidino-2-phenylindole nuclear stain (Beckman Coulter, Inc.). Fluorescence-activated cell sorting (FACS) analysis was done on stained cells immediately with a flow cytometer (Cell Lab Quanta SC, Beckman Coulter).

Apoptosis assay. For measuring apoptosis, transfected cells were dual stained with the viability dye 7-aminoactinomycin D (7-AAD) and Annexin V-FITC using Annexin V-FITC/7-AAD kit (Beckman Coulter) according to the manufacturer's protocol.

Figure 2. Effect of genistein and TSA on MCM gene family. A and B, relative mRNA expression levels of MCM2 family members in PC3 and LNCaP cells as assessed by qRT-PCR after treatment with genistein (G) and TSA alone or in combination. mRNA levels of MCM members were significantly downregulated after treatment compared with vehicle control–treated cells. *, statistically significant at P ≤ 0.05. C and D, Western blot analysis showing downregulation of MCM members at the protein level after genistein, TSA, or cotreatment in PC3 and LNCaP cells. Control, vehicle control; 25G, 25 μmol/L genistein; 25G+T, 25 μmol/L genistein + 100 ng TSA; 50G, 50 μmol/L genistein; 50G+T, 50 μmol/L genistein + 100 ng TSA.
Stained cells were immediately analyzed by flow cytometry (Cell Lab Quanta SC).

**miRNA transfection.** The day before transfection, cells were plated in growth medium without antibiotics at a density of approximately 40% to 50%. Transient transfection of miR-1296 at 50 nmol/L concentration was carried out with Lipofectamine 2000 according to the manufacturer’s protocol. Untransfected cells, mock, control miR, and a miR-1296 inhibitor were included as various controls. All miR transfectants were used after 72 h.

**Statistical analysis.** Statistical analysis was done using *StatView* version 5.0 for Windows as needed. The Student’s *t* test was used to compare the different groups. *P* values of <0.05 were regarded as statistically significant and is represented by an asterisk in the figures.

**Results**

**MCM2-MCM7 expression profile.** To examine the clinical relevance of the MCM gene family, their expression was analyzed in matched carcinoma and normal prostate tissue samples by Taqman quantitative real-time PCR analysis. Almost all the carcinoma samples showed higher expression of all the MCM gene family members compared with their matched normal samples. Expression of the MCM2 gene was highest among all other members in prostate cancer samples (Fig. 1A). Thus, we selected this gene to further confirm its overexpression in carcinomas by doing immunohistochemical staining on tissue microarrays containing human normal adjacent prostate tissue samples (*n* = 15), malignant tumor (*n* = 72), and metastatic carcinomas (*n* = 8). Immunohistochemistry on tissue microarrays revealed that MCM2 levels generally correlated with prostate cancer progression (Fig. 1B). In fact, the mean value of MCM2 expression in metastatic lesions (257 ± 5) was 64.3-fold greater than in normal samples (4 ± 2.5) and 2.0-fold greater than in primary tumors (131 ± 11; Fig. 1C). Staining was uniformly confined to the nuclear compartment, with rare cytoplasmic staining. The cells exhibiting staining were confined to the basal proliferating layer of the prostate epithelium, whereas stromal cells showed no staining. The luminal differentiated cells in nonmalignant glands were rarely positive for MCM2 staining. Prostate cancer is characterized by the loss of the basal cell layer (32). Therefore, MCM2 staining was evident in the luminal epithelial cells of malignant glands. Again, stromal cells were not positive for MCM2 staining. Representative pictures of MCM2 immunohistochemistry and quick score of immunostaining data are represented in Fig. 1B and C.

**Effect of genistein and TSA on the expression of MCM gene family.** Genistein and TSA alone or in combination significantly downregulated the relative expression levels of all the MCM gene family members compared with the vehicle control, although the degree of repression varied from one gene to another in both LNCaP and PC3 cells (Fig. 2A and B). To verify whether decreased transcription of these genes resulted in decreased levels of their respective proteins, we did Western blot analysis. Western blot analysis showed that the protein levels of all the MCM gene family were downregulated in genistein- or TSA-treated PC3 and LNCaP cells (Fig. 2C and D; Supplementary Fig. S1). These results agree with the quantitative RT-PCR (qRT-PCR) data and show that genistein and TSA downregulated transcription and translation of the MCM gene family.

**Knockdown of MCM2 by siRNA.** To understand the role of MCM2 downregulation in prostate cancer, we undertook a siRNA-mediated approach. Initially, three different sets of siRNA duplexes were tested to evaluate the target specificity and knockdown efficiency by quantitative real-time PCR (Fig. 3A). Two siRNA duplexes (S-1 and S-2) showing the highest knockdown efficiency at the protein level (Western blot analysis; Fig. 3B). To verify whether decreased transcription of these genes resulted in decreased levels of their respective proteins, we did Western blot analysis. Western blot analysis showed that the protein levels of all the MCM gene family were downregulated in genistein- or TSA-treated PC3 and LNCaP cells (Fig. 2C and D; Supplementary Fig. S1). These results agree with the quantitative RT-PCR (qRT-PCR) data and show that genistein and TSA downregulated transcription and translation of the MCM gene family.

**Induction of cell cycle arrest and apoptosis.** FACS analysis was done to test the effect of genistein and TSA on the cell cycle, and the results were compared with that of the siRNA duplexes. As summarized in Fig. 4, genistein or TSA treatment resulted in a significant decrease in the percentage of cells in the S phase of the cell cycle in PC3 (24% to 4%; Fig. 4A) and LNCaP (14% to 5%; Fig. 4B) cells compared with
vehicle-treated control. Knockdown by siRNA also showed a decrease in the percentage of cells in S phase from 22% to 2% (PC3 cells; Fig. 4B) or 14% to 6% (LNCaP cells; Fig. 4B) compared with the nonsilencing siRNA control. There was no change in the apoptotic population by genistein/TSA or siRNA duplexes (results not shown).

**Effect of genistein and TSA on the expression of genes that are important for loading of the MCM complex onto chromatin to facilitate DNA replication.** Central to the DNA replication licensing system is the formation of pre-RCs. The assembly of pre-RCs requires loading of the MCM2-MCM7 complex onto chromatin, which depends on CDT1, CDC7, and CDK2 genes. To check the effect of genistein/TSA on these genes, we did quantitative real-time PCR and Western blot assays. In PC3 cells, genistein and TSA alone or in combination significantly downregulated the expression of CDK2, CDC7, and CDT1 genes compared with the vehicle control. These results reveal that genistein and TSA have an inhibitory effect on the DNA replication licensing machinery.

miR-1296 expression is downregulated in prostate cancer. To examine the clinical relevance of miR-1296, its expression was analyzed in carcinoma ($n = 23$) and benign prostate hyperplasia (BPH; $n = 24$) tissue samples. Almost all the carcinoma samples showed highly reduced miR-1296 expression with respect to the BPH samples, and an overall lower relative average expression was observed in carcinoma compared with BPH samples (Fig. 6A). Transient transfection with miR-1296 at 50 nmol/L concentration in PC3 prostate cancer cells for 72 hours resulted in a 500,000-fold induction in miR-1296 expression in comparison with the control miR (data not shown).

**miR-1296 targets MCM2.** To find out whether MCM2 is a target of miR-1296, we used online search tools miRanda and identified a target site with high complementarity to miR-1296 in 3′-untranslated region (UTR) of MCM2. Overall, the alignment score is 140, with a perfect match to the seed heteramer (Fig. 6B, 1). MCM2 proved to be sensitive to miR-1296, and a significant decrease (51%) in MCM2 mRNA expression levels was observed in miR-1296–transfected cells (Fig. 6B, 2). Western blot analysis confirmed that MCM2 protein levels were also reduced in response to miR-1296 transfection (Fig. 6B, 3). Taken together, these data suggest that miR-1296 reduces MCM2 expression by inhibiting translation and/or causing mRNA instability/decay.
We also transfected cells with a miR-1296 inhibitor (anti-miR-1296; Applied Biosystems) designed specifically to bind and sequester mature miR-1296. The miR-1296 inhibitor helped preserve MCM2 expression at both the mRNA and the protein levels (Fig. 6B, 2 and 3). Taken together, these results indicate that the MCM2 transcript is a target of miR-1296.

**miR-1296 decreases the number of S-phase cells.** FACS analysis was done on miR-1296–transfected PC3 cells to test its effect on the cell cycle. miR-1296 caused a significant decrease in the percentage of S-phase cells (from 15% to 2%) compared with control miR, untransfected, and mock-transfected controls (Fig. 6C). We also did a quantitative real-time PCR assay to check the effect of genistein on miR-1296 expression. A synergistic effect of genistein on miR-1296 was observed, as there was an increase in miR-1296 expression of 3- to 5-fold with 25 or 50 μmol/L genistein (Fig. 6D).

**Discussion**

Cancer cells are highly proliferative; therefore, cell proliferation markers are very effective and attractive as cancer diagnostic markers (15). At present, MCM proteins are very good proliferation markers and are highly expressed in proliferating cells versus quiescent somatic cells (16), thus allowing for the identification of malignant cancer cells. In this study, we show that both genistein and TSA have an anti-MCM complex effect, causing the highest decrease in expression of MCM2 in prostate cancer cells. We also used a RNAi approach to target the MCM2 gene to examine its functional aspects in prostate cancer cells and compare the results with that of genistein and TSA. We found that miR-1296 is downregulated in prostate cancer and that MCM2 is one of its targets.

Genistein is believed to be a potent anticancer agent and has been shown to have antitumor effects in animal models (35). Various soy products containing genistein have been found to inhibit the growth of transplanted human prostate carcinoma, reduce the incidence of poorly differentiated prostate adenocarcinoma in a transgenic mouse model, and inhibit 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine–induced rat prostate cancer (36). Carcinogenesis or metastasis to the stomach, colon, bladder, and lung is also inhibited by genistein and related isoflavones (37–39). TSA, a histone deacetylase inhibitor and a potent anticancer drug, is currently undergoing clinical trials as a prostate cancer treatment but has high toxicity. Currently, there are no reports on the effect of genistein, a natural, nontoxic dietary isoflavone, or TSA on the MCM gene family. We found expression...
of MCM genes to be higher in prostate cancer compared with normal tissues. The MCM proteins are essential DNA replication factors that are highly expressed in malignant cancer and precancerous cells but downregulated in differentiated somatic cells (16, 26, 27, 32). Treatment with genistein or TSA alone or in combination significantly downregulated the expression of the MCM gene family in both androgen-dependent LNCaP or androgen-independent PC3 cells.

Drugs targeting MCM proteins are likely to prevent the proliferation of cancer cells by blocking replication licensing or DNA synthesis during the S phase (40). Our results show that genistein, TSA, and siRNA decreased the S phase of the cell cycle in both LNCaP and PC3 prostate cancer cells. It has been reported that when the negative regulator of replication licensing, geminin, is overexpressed in human primary cells, loading of the MCM complex to the chromatin is prevented and the cells are blocked in the G1 phase of the cell cycle (33, 34). A “licensing checkpoint” may exist in normal human cells, which blocks S-phase entry if replication licensing is incomplete or compromised (33, 34). Cancer cells, on the other hand, are defective in this checkpoint. When treated with anti-MCM drugs that compromise DNA replication licensing, they enter the S phase with underlicensed G1 chromatin and are eliminated (40).

The loading of the MCM2-MCM7 complex is dependent on CDT1 (licensing; refs. 5, 6), CDC7, and CDK2 that recruit additional factors to pre-RCs, resulting in the formation of pre-ICs (7, 8). In addition, CDC7 and CDK2 activate the putative MCM2-MCM7 helicase, which, together with pre-IC formation, results in recruitment of DNA polymerases and initiation of DNA replication. Our results show that both genistein and TSA alone or in combination significantly downregulated the expression of CDK2, CDC7, and CDT1 genes at the mRNA and protein levels compared with the vehicle control. These results show that genistein and TSA have inhibitory effects on the DNA replication licensing machinery in prostate cancer.

miRs are a class of naturally occurring small noncoding RNAs that control gene expression by binding to sites in the 3′-UTRs of target transcripts, resulting in translational arrest and, in some instances, transcript degradation (41). Recent estimates suggest that one third of human miRNAs may be regulated by miRs (42). Studies have found that miR expression patterns are significantly different in normal and neoplastic tissues, suggesting that miRs may play a role...
in tumorigenesis (43). In human cancers, some miRs may have an oncogenic function due to their overexpression in malignant tissue, whereas certain miRs may act as tumor suppressor genes because of decreased expression (43). Differential expression of miRs has been reported in prostate cancer (44). We searched the online database to look for miRs that regulate MCM2 and found that miR-1296 has a perfect seed complimentary sequence in the 3’-UTR of the MCM2 gene. The expression of miR-1296 was significantly lower in prostate tumors than BPH tissues. Transfection of miR-1296 in PC3 prostate cancer cells decreased the expression of MCM2 mRNA and protein. To further validate that miR-1296 inhibits MCM2 expression in prostate cancer, we transfected PC3 cells with the miR-1296 inhibitor, which is designed specifically to bind and sequester the mature miR-1296 sequence. MCM2 mRNA expression in miR-1296 inhibitor–transfected cells was upregulated relative to control miR–tansfected cells. MCM2 protein expression was also upregulated in miR-1296 inhibitor–transfected cells. These results show that inhibition of miR-1296 upregulated MCM2 mRNA and protein expression in prostate cancer cells, confirming that miR-1296 inhibits expression of the MCM2 gene. We also observed a significant decrease in S-phase cells in response to miR-1296 compared with controls. Genistein had a positive effect on miR-1296, as there was an increase of 3- to 5-fold in miR-1296 expression compared with vehicle control.

MCM proteins are sensitive markers for early cancer diagnosis. Their essential role in cancer cell proliferation makes them attractive therapeutic drug targets. Because cell proliferation is a highly conserved cellular process, anti-MCM drugs may be effective against a broad spectrum of cancers, regardless of their organ or tissue of origin. Our study clearly showed that genistein, a natural, nontoxic dietary isoflavone, has anti-MCM effects in prostate cancer. We also found that MCM2 is a target of miR-1296. To our knowledge, this is the first report showing the effect of genistein on the MCM licensing gene family in prostate cancer. TSA is currently undergoing clinical trials as a prostate cancer treatment but has high toxicity. Genistein, a natural, nontoxic dietary isoflavone, may be an advantageous therapeutic agent for treating prostate cancer. The use of RNAi is currently being implemented as a gene-specific approach for molecular medicine. By the same principle, the specific downregulation of oncogenes by miR may contribute to the growing therapeutic potential that small RNA-based drugs have in the treatment of cancer and other diseases. It is possible that stimulation of silenced miRs by drug treatment may lead to the downregulation of target oncogenes, thereby contributing to novel therapeutic approaches in the treatment of prostate cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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