miR-1226 targets expression of the mucin 1 oncoprotein and induces cell death

CAINING JIN, HASAN RAJABI and DONALD KUFE

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA

Received March 11, 2010; Accepted April 8, 2010

DOI: 10.3892/ijo_00000653

Abstract. The MUC1 oncoprotein is aberrantly overexpressed in human carcinomas and hematologic malignancies. MicroRNAs (miRNAs) have been implicated in the suppression and induction of oncogenesis. The present studies demonstrate that the MUC1 mRNA 3' untranslated region (3'UTR) contains a highly conserved motif for binding of a novel miRNA, miR-1226, that has no known targets. The results show that miR-1226 is expressed in human breast cancer cell lines and non-malignant mammary epithelial cells. We also show that miR-1226 interacts with the MUC1 mRNA 3'UTR and that miR-1226 downregulates endogenous MUC1 protein levels. Consistent with miR-1226-induced downregulation of MUC1 expression, the results demonstrate that miR-1226 induces i) an increase in reactive oxygen species, ii) loss of the mitochondrial transmembrane potential, and iii) a decrease in cell survival. These findings indicate that expression of the MUC1 oncoprotein is downregulated by miR-1226 and that miR-1226 thereby functions as a tumor suppressor by promoting the induction of cell death.

Introduction

The mucin 1 (MUC1) oncoprotein is aberrantly expressed in most human carcinomas and certain hematologic malignancies (1). The upregulation of MUC1 expression has been attributed to activation of the *MUC1* gene (1), and amplification and rearrangements of the *MUC1* locus (2,3). Following transcription, MUC1 is translated as a single polypeptide, undergoes autocleavage into two subunits, which in turn form a stable heterodimer (1). The MUC1 N-terminal subunit (MUC1-N) contains a glycosylated tandem repeat structure that is characteristic of mucins (1). By contrast, the MUC1 C-terminal subunit functions as a transmembrane receptor that also tethers MUC1-N to the cell surface and interacts with members of the receptor tyrosine kinase family (1,4). Significantly, the MUC1-C 72 amino acid cytoplasmic domain (MUC1-CD) is sufficient to induce transformation (5,6). In this regard, MUC1-CD interacts with diverse effectors, such as ß-catenin (6,7), p53 (8), IκB kinase (9) and NF-κB p65/RelA (10) that contribute to the induction of transformation. The overexpression of MUC1 in malignant cells is associated with accumulation of MUC1-C in the cytoplasm and targeting of MUC1-C to the nucleus (11), where it participates in the regulation of target genes activated by diverse transcription factors (8,10,12,13). MUC1-C is also targeted to the mitochondrial outer membrane, where it protects against stress-induced loss of the mitochondrial transmembrane potential (14,15). In concert with these observations, overexpression of MUC1-C blocks the induction of apoptosis and necrosis in the response of human cancer cells to genotoxic stress (14), reactive oxygen species (16,17), hypoxia (18), glucose deprivation (19) and activation of death receptors (20). These findings have provided substantial evidence that dysregulation of MUC1 expression is of importance to the malignant phenotype.

MicroRNAs (miRNAs) are a class of small (~22 nucleotides) non-coding RNAs that posttranscriptionally regulate gene expression (21). miRNAs interact in a sequence-specific manner with the 3' untranslated region (3'UTR) or, in some settings, with amino acid coding regions of their mRNA targets (22). Expression of the target is thereby directed by miRNA-induced mRNA degradation or inhibition of translation (23). The identification of aberrant patterns of miRNA expression in human cancers has indicated that miRNAs can function in oncogenesis and as tumor suppressors (24). Mechanisms that have been attributed to aberrant miRNA expression in malignant cells include chromosomal alterations, epigenetic modifications, mutations, polymorphisms and defective miRNA biogenesis (24). Dysregulation of miRNA expression has been found in diverse carcinomas and hematologic malignancies, and has been linked to alterations in cell growth, survival and metastasis (24,25). For example, miR-15 and miR-16 were first identified as regulators of Bcl-2 and thereby the apoptotic response (26). Other miRNAs have been implicated in regulating TRAIL-induced activation of the extrinsic apoptotic pathway (25). Moreover, the miR-34 family is induced by p53 and regulates genes

Correspondence to: Dr Donald Kufe, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA
E-mail: donald_kufe@dfci.harvard.edu

Abbreviations: miRNA, microRNA; MUC1, mucin 1; MUC1-N, MUC1 N-terminal subunit; MUC1-C, MUC1 C-terminal subunit, MUC1-CD, MUC1 cytoplasmic domain

Key words: microRNA, MUC1, reactive oxygen species, mitochondria, apoptosis, necrosis

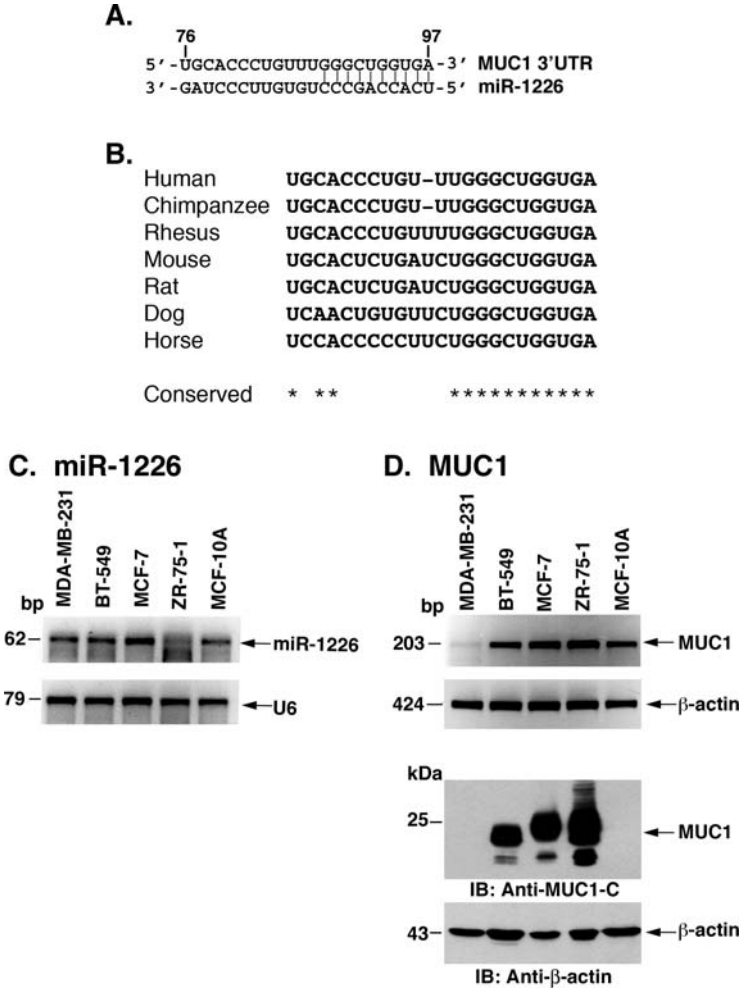

Figure 1. Human MUC1 mRNA 3'UTR contains a miR-1226 binding element. (A) Identification of a putative miR-1226 binding sequence in the MUC1 3'UTR at 76-97 bp. Ten bases of the MUC1 sequence are perfect matches for the miR-1226 5' region. (B) Comparison of the MUC1 binding element among mammals demonstrates a high degree of conservation as denoted by the asterisks. (C) miR-1226 levels were determined by RT-PCR for the indicated cell lines. U6 expression was used as a control. (D) MUC1 mRNA (upper panels) and protein (lower panels) levels were determined by RT-PCR and immunoblotting, respectively. ß-actin expression was used as controls.

involved in the p53-dependent growth arrest and apoptotic responses (27,28). Other work has demonstrated that miR-125b is a negative regulator of p53 expression and the induction of apoptosis (29). These findings have collectively provided convincing support for the importance of miRNAs in regulating the apoptotic response of cancer cells.

The present work demonstrates that miR-1226, a miRNA with no known target transcripts, is a novel negative regulator of MUC1 expression. The results also demonstrate that miR-1226-induced downregulation of MUC1 is associated with death of MUC1-positive breast cancer cells.

Materials and methods

Cell culture. Human MCF-7 and MDA-MB-231 breast cancer and 293 embryonic kidney cells (ATCC) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) containing 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. Human ZR-75-1 breast cancer cells (ATCC) were grown in RPMI-1640 medium supplemented with 10% FBS, antibiotics and L-glutamine. Human BT-549 breast cancer cells were cultured in ATCC-formulated RPMI-1640 medium supplemented with 0.023 IU/ml insulin and 10% FBS. Human MCF-10A mammary epithelial cells (ATCC) were cultured in mammary epithelial cell growth medium (Lonza). Transfection of MCF-7 cells with small interfering RNA (siRNA) pools (Dharmacon) or miRNA mimics (Qiagen) were performed in the presence of siPORT NeoFX Transfection Agent (Ambion). Other cell lines were transfected in the presence of Lipofectamine 2000 (Invitrogen).

RT-PCR. Total RNA was isolated from cells using the mirVana miRNA Isolation Kit (Ambion). cDNAs were synthesized using the Ncode miRNA first-strand cDNA synthesis kit (Invitrogen). Expression of miR-1226 and MUC1 was analyzed with Taq DNA polymerase (Qiagen).

Immunoblot analysis. Lysates from subconfluent cells were prepared as described (14) and subjected to immunoblotting with anti-MUC1-C (Ab5; NeoMarkers) and anti-ß-actin (AC-15; Sigma-Aldrich). Immune complexes were detected with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham Biosciences).

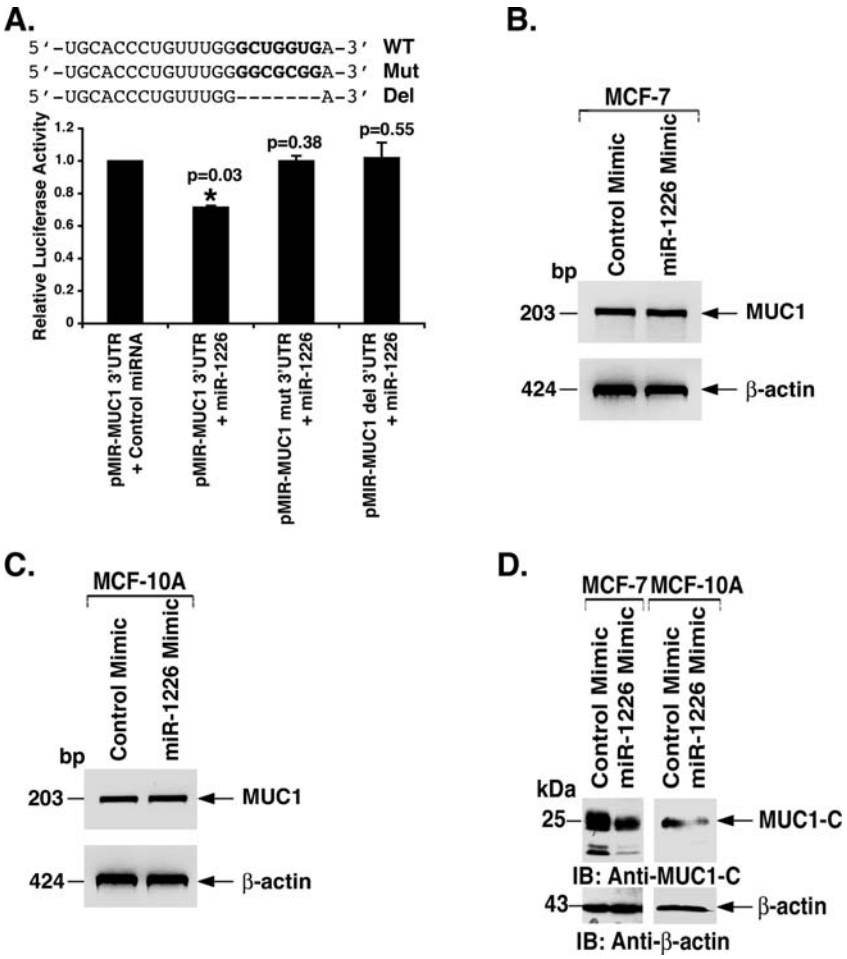

Figure 2. miR-1226 interacts with the MUC1 mRNA 3'UTR. (A) Wild-type MUC1 mRNA 3'UTR was cloned into the pMIR-luciferase vector (pMIR-MUC1 3'UTR). The indicated MUC1 sequence was then mutated (pMIR-MUC1 mut3'UTR) or deleted (pMIR-MUC1 del3'UTR) in the pMIR-MUC1 3'UTR vector (upper panel). The 293 cells were transfected with pMIR-MUC1 3'UTR, TK-Renilla-Luc, and 100 nM control mimic or miR-1226 mimic. At 48 h after transfection, the cells were analyzed for luciferase activity. The results are expressed as relative luciferase activity (mean ± SD of three determinations) as compared to that obtained with cells expressing pMIR-MUC1 3'UTR and the control mimic (assigned a value of 1) (lower panel). The p-value was determined using the Student's t-test. (B and C) MCF-7 (B) and MCF-10A (C) cells were transfected with 100 nM control or miR-1226 mimics. At 60 h after transfection, miR-1226 (left) and MUC1 mRNA (right) levels were determined by RT-PCR. (D) MCF-7 and MCF-10A cells were transfected with 100 nM control or miR-1226 mimics. At 60 h after transfection, the cells were analyzed for MUC1-C and ß-actin levels by immunoblotting. As determined by densitometric scanning of the signals, the decrease in MUC1-C expression in the MCF-7 and MCF-10A cells was 51 and 63%, respectively.

Luciferase reporter assays. The human MUC1 3'UTR was ligated into the pMIR-REPORT expression vector (Ambion). Mutation and deletion of the MUC1 3'UTR at the miR-1226 binding site was performed using the QuickChange XL site-directed mutagenesis kit (Stratagene). Cells were cotransfected with the pMIR-MUC1 3'UTR vectors, pRL-TK (Promega) and the miR-1226 mimic or negative control mimic in the presence of Lipofectamine 2000. At 48 h after transfection, the cells were analyzed for firefly and Renilla luciferase activities using the Dual Luciferase Assay Kit (Promega).

Measurement of ROS levels. Cells were incubated in PBS containing 10 μM DCFH-AM for 30 min at 37˚C in the dark to assess H_2O_2-mediated oxidation of DCFH-AM to the fluorescent DCF. Fluorescence of DCF was measured by flow cytometry.

Measurement of the mitochondrial transmembrane potential. Cells were incubated in PBS containing 500 ng/ml rhodamine 123 (Molecular Probes) for 20 min at 37˚C in the dark. Samples were washed in PBS and analyzed by flow cytometry.

Assessment of cell death. Cells were incubated in binding buffer containing FITC-conjugated Annexin V and propidium iodide (PI) (BD Pharmingen) for 15 min at room temperature in the dark and then analyzed by flow cytometry. For clonogenic survival assays, cells were seeded in six-well plates. After two weeks, colonies were stained with crystal violet and counted as positive when containing over 50 cells.

Results

Human MUC1 3'UTR contains a putative element for miR-1226 binding. To assess the possible regulation of MUC1 expression by miRNAs, we searched the human MUC1 321 base-pair (bp) 3'UTR for potential miRNA binding sites using TargetScanHuman 5.1 (30-32). A putative response element for miR-1226 was identified at 76-97 bp downstream of the

stop-codon (Fig. 1A). Searches for miR-1226 targets by TargetScan and miRanda (33) confirmed that the MUC1 3'UTR contains a potential miR-1226 binding site. In this regard, the MUC1 3'UTR sequence GGGCUGGUGA has perfect complimentarity with bases 1 through 10 counting from the 5' end of miR-1226 (Fig. 1A). MUC1 homologs are restricted to mammalian species (34). Highly conserved putative miR-1226 response elements were found in the human MUC1 3'UTR and that of other vertebrates, indicating that miR-1226 may be of importance as a regulator of MUC1 expression (Fig. 1B). The precursor sequence for miR-1226 is localized on chromosome 3 (35). Otherwise, there are no published reports on miR-1226 expression or function. Analysis of human MDA-MB-231, BT-549 and MCF-7 breast cancer cell lines demonstrated expression of miR-1226 (Fig. 1C). By contrast, ZR-75-1 breast cancer cells had somewhat lower levels of miR-1226 expression (Fig. 1C). miR-1226 was also detectable in non-malignant human MCF-10A mammary epithelial cells (Fig. 1C). MUC1 mRNA levels were lower in MDA-MB-231 cells (Fig. 1D, upper panels). For comparison, MUC1 protein levels were elevated in ZR-75-1 cells as compared to that in MCF-7 and BT-549 cells (Fig. 1D, lower panels). Moreover, MUC1 expression was relatively lower in MDA-MB-231 and MCF-10A cells, although detectable upon longer radiographic exposures (Fig. 1D, lower panels; data not shown).

MUC1 expression is suppressed by miR-1226. To assess binding of miR-1226 to the putative target sequence, the region containing the human MUC1 3'UTR was cloned into the pMIR-luciferase reporter (pMIR-MUC1 3'UTR). As controls, the MUC1 3'UTR was mutated (4-base mismatch; pMIR-MUC1 mut3'UTR) or deleted (pMIR-MUC1 del3'UTR) in the GCUGGUG sequence (Fig. 2A, upper). Transfection of 293 cells with pMIR-MUC1 3'UTR and a miR-1226 mimic was associated with downregulation of luciferase activity (Fig. 2A, lower). Compared to wild-type pMIR-MUC1 3'UTR, expression of the mutated pMIR-MUC1 mut3'UTR vector was unaffected by the miR-1226 mimic (Fig. 2A, lower). Deletion of GCUGGUG in pMIR-MUC1 del3'UTR also abrogated downregulation of expression by the miR-1226 mimic (Fig. 2A, lower), indicating that the predicted response element is essential for direct binding of miR-1226. To determine whether miR-1226 regulates endogenous MUC1 expression by decreasing mRNA stability, we studied MCF-7 cells transfected to overexpress the control or miR-1226 mimics. RT-PCR analysis demonstrated that miR-1226 has little if any effect on MUC1 mRNA levels (Fig. 2B). Similar findings were obtained with MCF-10A cells expressing control and miR-1226 mimics (Fig. 2C). By contrast, expression of MUC1-C protein was suppressed by miR-1226 in both MCF-7 and MCF-10A cells (Fig. 2D). These findings indicate that miR-1226 interacts with the MUC1 3'UTR and suppresses MUC1 translation.

miR-1226 overexpression increases ROS levels and induces loss of mitochondrial transmembrane potential. Previous work has demonstrated that MUC1-C suppresses intracellular oxidant levels (16-19). Consistent with these findings, expression of the miR-1226, and not the control, mimic in

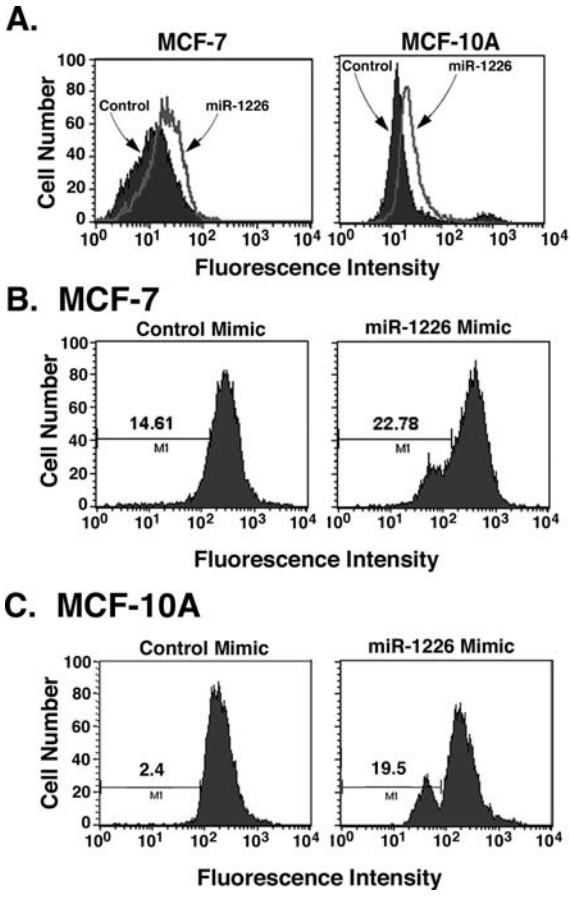

Figure 3. miR-1226 disrupts redox balance and induces loss of mitochondrial transmembrane potential ($\Delta\psi m$). (A-C) MCF-7 and MCF-10A cells were transfected with 100 nM control or miR-1226 mimics. At 60 h after transfection, the cells were incubated with DCFH-AM and fluorescence of oxidized DCF was assessed by flow cytometry (A). The cells were also stained with Rhodamine 123 and analyzed by flow cytometry (B and C).

MCF-7 cells was associated with an increase in ROS levels (Fig. 3A, left). Expression of the miR-1226 mimic similarly disrupted redox balance in MCF-10A cells (Fig. 3A, right), indicating that this response occurs in both malignant and non-malignant mammary epithelial cells. Other studies have shown that MUC1 protects against stress-induced loss of the mitochondrial transmembrane potential ($\Delta\psi m$) (14,15). Significantly, downregulation of MUC1 and disruption of redox balance by miR-1226 was also associated with loss of $\Delta\psi m$ in both MCF-7 and MCF-10A cells (Fig. 3B and C).

Overexpression of miR-1226 decreases cell survival. To determine whether miR-1226-induced disruption of redox balance and loss of $\Delta\psi m$ affects survival, cells expressing the control and miR-1226 mimics were first monitored for effects on growth. As compared to the controls, growth of MCF-7 (Fig. 4A) and MCF-10A (Fig. 4B) cells was decreased by expression of the miR-1226 mimic. In support of MUC1 as a target, silencing MUC1 expression with a MUC1 siRNA pool was associated with slowing of MCF-7 cell growth (Fig. 4C). Similar effects were observed in MCF-10A cells (Fig. 4D).

Assessment of cell survival by colony formation assays further demonstrated a substantial decrease in MCF-7 cells

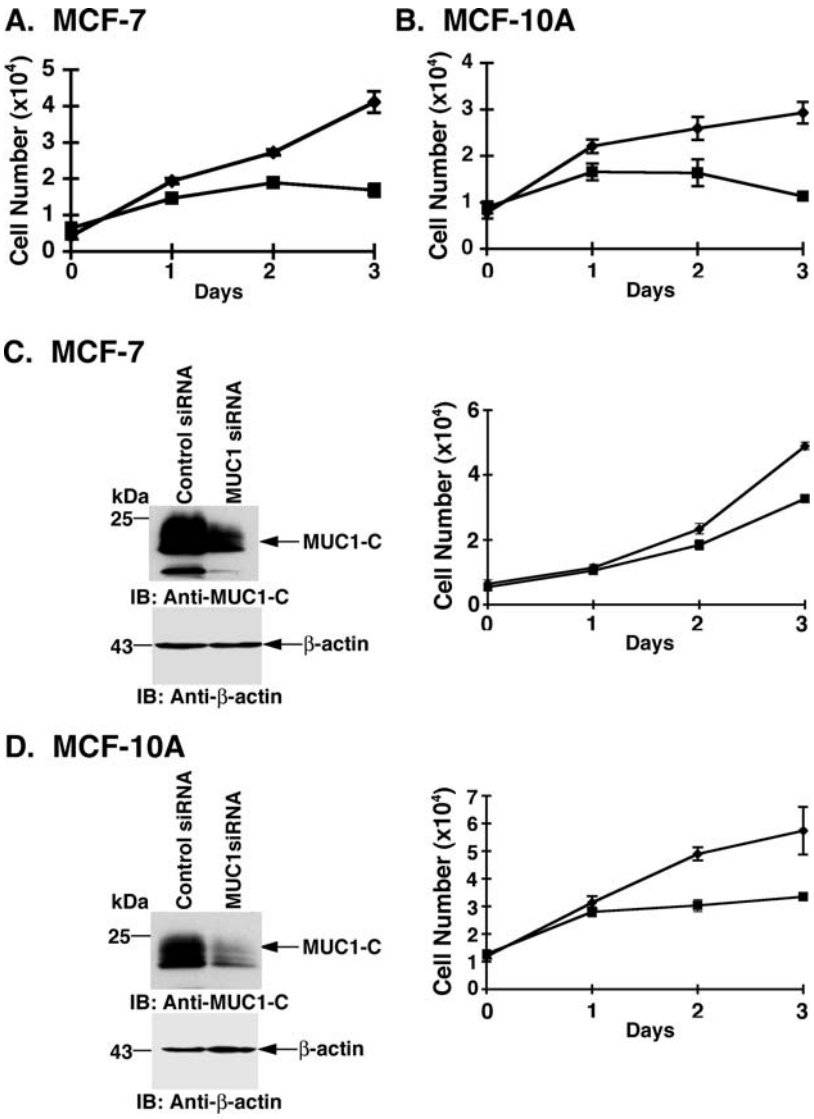

Figure 4. miR-1226 inhibits MCF-7 and MCF-10A cell growth. (A and B) MCF-7 (A) and MCF-10A (B) cells were transfected with 100 nM control (diamonds) or miR-1226 (squares) mimics. Viable cell number was determined at the indicated times by trypan blue exclusion. (C and D) MCF-7 (C) and MCF-10A (D) cells were transfected with control or MUC1 siRNA pools. At 72 h after transfection, cell lysates were immunoblotted with the indicated antibodies (left). As determined by densitometric scanning of the signals, the decrease in MUC1-C expression in the MCF-7 and MCF-10A cells was 43 and 48%, respectively. Growth of control siRNA- (diamonds) and MUC1siRNA- (squares) transfected cells was determined by trypan blue staining (right).

expressing miR-1226 as compared to the control mimic (Fig. 5A, left). Analysis of repetitive experiments confirmed that miR-1226 decreases MCF-7 colony formation by nearly 75% (Fig. 5A, right). Similar effects were observed with MCF-10A cells expressing miR-1226 (Fig. 5B. left) with a decrease of over 50% in colony formation (Fig. 5B, right). In addition, miR-1226 had little if any effect on growth and survival of MUC1-negative 293 cells (Fig. 5C).

miR-1226 induces late apoptosis/necrosis. MUC1 attenuates the apoptotic and necrotic death responses to oxidative stress (16-19). To determine the basis for miR-1226-induced death, cells were visualized by bright field microscopy and after staining with DAPI. Expression of the miR-1226 mimic was associated with rounding-up of the MCF-7 cells and nuclear condensation, consistent with an apoptotic response (Fig. 6A). Similar results were obtained with MCF-10A cells expressing the miR-1226 mimic (Fig. 6B). To extend and quantitate these observations, the cells were stained with PI/Annexin V and analyzed by flow cytometry. For MCF-7 cells expressing the miR-1226 mimic, 44% were Annexin V-positive as compared to 14% for those expressing the control (Fig. 6C, upper panels). These results were confirmed in repetitive experiments (Fig. 6C, lower panel). In addition, about half of the Annexin V-positive cells had increased PI uptake, consistent with the induction of late apoptosis/necrosis. Expression of the miR-1226 mimic in MCF-10A cells was also associated with a significant increase in Annexin V-positive cells, many of which exhibited PI uptake (Fig. 6D), indicating the induction of predominantly a late apoptotic/necrotic response.

Discussion

miR-1226 downregulates MUC1 expression. MUC1 is aberrantly overexpressed in diverse human malignancies (1).

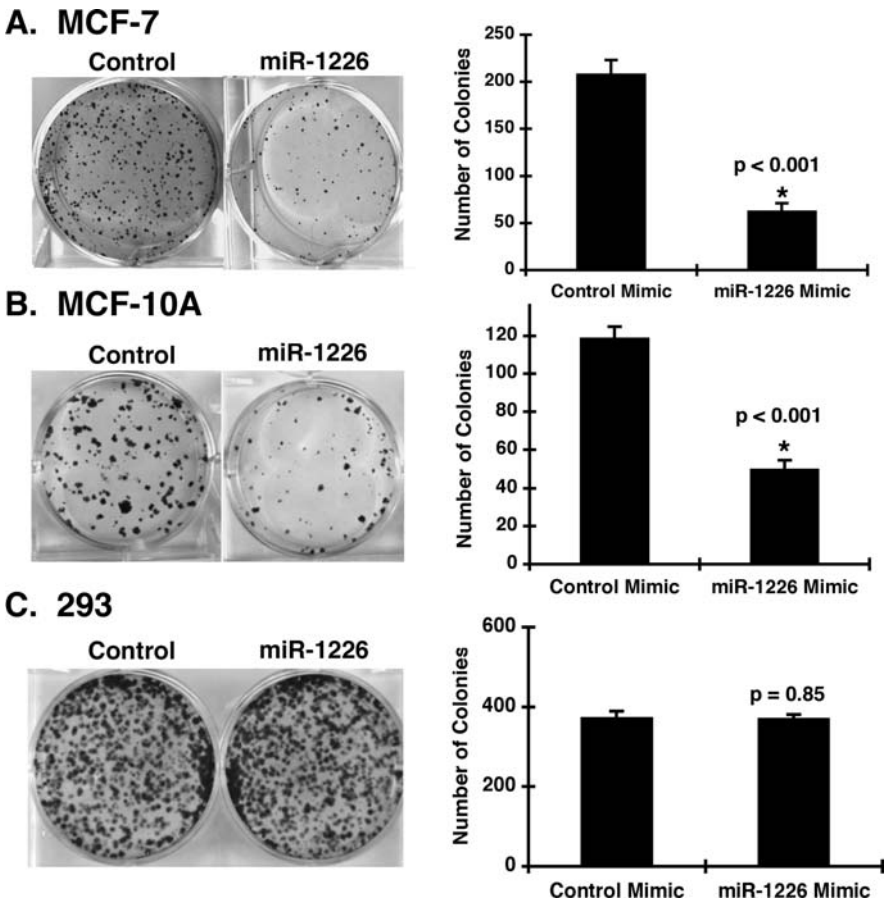

Figure 5. miR-1226 suppresses MCF-7 and MCF-10A cell survival. (A-C) The indicated cells were transfected with 100 nM control or miR-1226 mimics. At 24 h after transfection, cells were trypsinized and replated into 6-well plates (1000 cells per well). Colonies were stained with crystal violet at 14 days and photographed (left). The number of colonies is expressed as the mean ± SD of three determinations (right).

Constitutive activation of the STAT3 and NF-κB pathways in human cancers contributes, at least in part, to aberrant induction of the MUC1 promoter (10,36). In addition, recent studies have demonstrated that MUC1 expression is regulated by post-transcriptional mechanisms involving interactions of the 321 bp MUC1 3'UTR with miR-145 and miR-125b (37,38). In the present work, we found that the MUC1 3'UTR contains a GGGCUGGUGA sequence that has perfect complimentarity with the 5' region of miR-1226. As determined by site-directed mutagenesis, we also found that miR-1226 interacts with this motif. Little is known about miR-1226 other than the pre-miR-1226 locus resides on chromosome 3 (35). The results shown here demonstrate that miR-1226 is expressed in human breast cancer cells and non-malignant mammary epithelial cells. Studies using human prostate and other types of cancer cells also identified miR-1226 expression (data not shown), indicating that this miRNA may be widely detectable in malignant cells that overexpress MUC1. In addition, analysis of human 3'UTRs identified putative miR-1226 binding sequences with perfect 7-bp matches in 29 additional genes (Table I), one of which, *BCL2L1* encoding Bcl-xL is known to be linked to MUC1 signaling pathways (10). Whereas further experimentation is underway to determine if miR-1226 regulates expression of these genes, in the present focus on MUC1, we found that miR-1226 confers a decrease in MUC1 protein levels. These findings and those of MUC1 promoter activation (1) indicate that overexpression of MUC1 in breast cancer cells is a function of multiple events that are dysregulated at both the transcriptional and post-transcriptional levels.

miR-1226 induces cell death by promoting apoptosis/necrosis. miR-15 and miR-16 downregulate expression of the anti-apoptotic Bcl-2 protein and thereby contribute to activation of the intrinsic apoptotic pathway (39). Other miRNAs have been linked to the downregulation of anti-apoptotic factors, for example, miR-29 and the Mcl-1 protein (25). The MUC1-C subunit is targeted to the mitochondrial outer membrane, where it attenuates loss of $\Delta\Psi m$ in the response to DNA damage, ROS and other forms of stress that activate the intrinsic apoptotic pathway (1,14,15). MUC1-C has also been implicated in suppressing redox imbalance (16), for example as a result of mitochondrial dysfunction and increases in ROS production (40,41). In concert with these findings, miR-1226-induced downregulation of MUC1 expression was associated with increases in ROS and loss of $\Delta\Psi m$. Moreover, miR-1226 expression in MCF-7 cells resulted in induction of a late apoptotic/necrotic response and loss of clonogenic survival. These effects of miR-1226 can be attributed, at least in part, to downregulation of MUC1 expression. In this regard, miR-1226 had little if any effect on 293 cells, which are null for MUC1 expression.

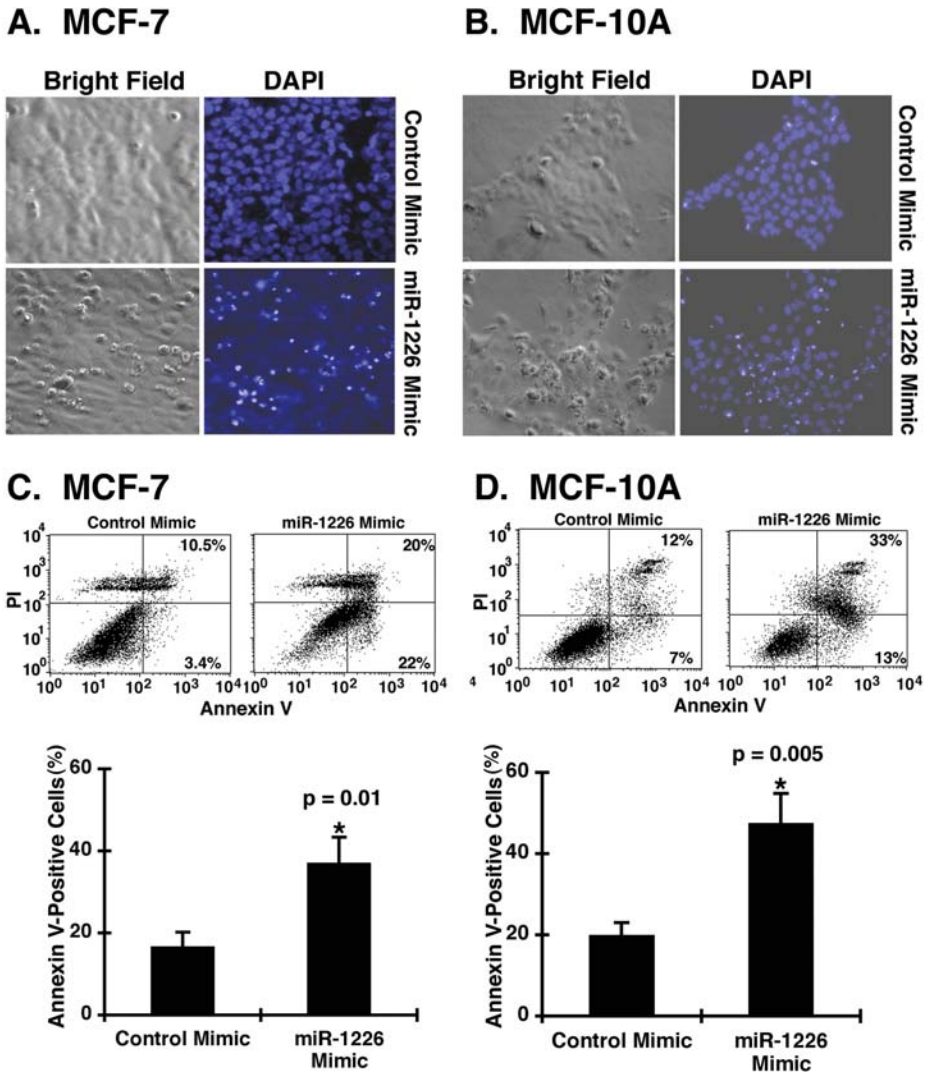

Figure 6. miR-1226 induces late apoptotic/necrotic cell death. (A-D) MCF-7 and MCF-10A cells were transfected with 100 nM control or miR-1226 mimics. (A and B) At 60 h after transfection, photomicrographs of the cells were taken (left) and the cells were stained with DAPI (right). (C and D) At 72 h after transfection, the cells were stained with PI and Annexin V, and analyzed by flow cytometry (upper panels). The results are expressed as the percentage of Annexin V-positive cells (mean ± SD of three determinations) (lower panels).

Table I. Genes with putative miR-1226 binding motifs.

PPFIBP1	MAT2A	NRP1	DLG2	NAT13	CREBBP
CRTAP	SOX11	KCNMA1	FBXO11	TFEC	FOSB
OGT	RPS6KB1	MUC1	IL11RA	MORF4L1	FBXW7
CAMK2G	CCND2	TUSC2	RAB15	PLSCR3	ZMIZ1
OTUB1	ZNF276	MAGF	EIF2C1	BCL2L1	FBXW2

Genes with putative miR-1226 binding motifs (perfect 7 bp complimentarity) in their 3'UTR, as determined by TargetScan and miRanda.

Nonetheless, these results do not exclude the possibility that the pro-death effects of miR-1226 are mediated by suppression of MUC1 in association with the downregulation of other genes. For example, the *BCL2L1* gene encodes the anti-apoptotic Bcl-xL protein and has a miR-1226 binding sequence in its 3'UTR (Table I). Indeed, preliminary results indicate that expression of the miR-1226 mimic decreases BCL2L1 mRNA levels (data not shown). Thus, it is possible that miR-1226-induced downregulation of MUC1 with disruption of redox balance initiates a pro-death response that is further promoted by decreases in the Bcl-xL protein.

Is miR-1226 a tumor suppressor in human cancers? Expression of miR-15 and miR-16 is suppressed in chronic lympho-

cytic leukemia cells that have upregulation of Bcl-2 levels (26,39,42). Moreover, miR-29 is decreased in cancer cells with overexpression of Mcl-1 (25,43). Our studies in non-malignant MCF-10A mammary epithelial cells demonstrate that miR-1226 downregulates MUC1 protein levels, indicating that this response is not limited to breast cancer cells. miR-1226 also induced i) increases in ROS, ii) loss of $\Delta\Psi m$, and iii) MCF-10A cell death. These results suggest that miR-1226 has a tumor suppressor function and that this role may be abrogated in breast cancer cells. Indeed, the relatively lower levels of miR-1226 in ZR-75-1 cells, compared to that in MCF-10A cells, suggest that overexpression of MUC1 may be due to suppression of miR-1226. Thus, constitutive activation of the *MUC1* promoter in breast cancer cells (10,36) is likely one factor in dictating MUC1 mRNA levels and decreases in miR-1226 may further contribute to the overexpression of MUC1. In addition, in the absence of constitutive *MUC1* promoter activation as found in MCF-10A cells (10), miR-1226 may be functional as a tumor suppressor in preventing aberrant upregulation of MUC1 and a malignant phenotype. If such a model proves correct, then miR-1226 levels will be of importance to the regulation of MUC1 expression and thereby MUC1-induced transformation. Analysis of miRNA expressional databases from human breast cancers will be needed to further define the prognostic significance of miR-1226. However, given the previous lack of functional information regarding miR-1226, to our knowledge none of the publicly available databases have included analyses of miR-1226 expression. The present findings could thus provide the experimental basis for analyzing miR-1226 levels in databases from human breast and other types of cancers that overexpress the MUC1 oncoprotein.

Acknowledgements

This work was supported by Grant CA97098 and CA42802 awarded by the National Cancer Institute. The authors acknowledge Dr Isidore Rigoutsos, Thomas Jefferson University Medical College, for his critical reading of the manuscript and his insightful suggestions.

References

1. Kufe D: Functional targeting of the MUC1 oncogene in human cancers. Cancer Biol Ther 8: 1201-1207, 2009.
2. Kawano T, Ahmad R, Nogi H, Agata N, Anderson K and Kufe D: MUC1 oncoprotein promotes growth and survival of human multiple myeloma cells. Int J Oncol 33: 153-159, 2008.
3. Dyomin VG, Palanisamy N, Lloyd KO, *et al*: MUC1 is activated in a B-cell lymphoma by the t(1;14)(q21;q32) translocation and is rearranged and amplified in B-cell lymphoma subsets. Blood 95: 2666-2671, 2000.
4. Ramasamy S, Duraisamy S, Barbashov S, Kawano T, Kharbanda S and Kufe D: The MUC1 and galectin-3 oncoproteins function in a microRNA-dependent regulatory loop. Mol Cell 27: 992-1004, 2007.
5. Li Y, Liu D, Chen D, Kharbanda S and Kufe D: Human DF3/MUC1 carcinoma-associated protein functions as an oncogene. Oncogene 22: 6107-6110, 2003.
6. Huang L, Chen D, Liu D, Yin L, Kharbanda S and Kufe D: MUC1 oncoprotein blocks GSK3ß-mediated phosphorylation and degradation of ß-catenin. Cancer Res 65: 10413-10422, 2005.
7. Yamamoto M, Bharti A, Li Y and Kufe D: Interaction of the DF3/MUC1 breast carcinoma-associated antigen and ß-catenin in cell adhesion. J Biol Chem 272: 12492-12494, 1997.
8. Wei X, Xu H and Kufe D: Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response. Cancer Cell 7: 167-178, 2005.
9. Ahmad R, Raina D, Trivedi V, *et al*: MUC1 oncoprotein activates the IκB kinase ß complex and constitutive NF-κB signaling. Nat Cell Biol 9: 1419-1427, 2007.
10. Ahmad R, Raina D, Joshi MD, Kawano T, Kharbanda S and Kufe D: MUC1-C oncoprotein functions as a direct activator of the NF-kappaB p65 transcription factor. Cancer Res 69: 7013-7021, 2009.
11. Leng Y, Cao C, Ren J, *et al*: Nuclear import of the MUC1-C oncoprotein is mediated by nucleoporin NUP62. J Biol Chem 282: 19321-19330, 2007.
12. Wei X, Xu H and Kufe D: MUC1 oncoprotein stabilizes and activates estrogen receptor α. Mol Cell 21: 295-305, 2006.
13. Wei X, Xu H and Kufe D: Human mucin 1 oncoprotein represses transcription of the p53 tumor suppressor gene. Cancer Res 67: 1853-1858, 2007.
14. Ren J, Agata N, Chen D, *et al*: Human MUC1 carcinoma-associated protein confers resistance to genotoxic anti-cancer agents. Cancer Cell 5: 163-175, 2004.
15. Ren J, Bharti A, Raina D, Chen W, Ahmad R and Kufe D: MUC1 oncoprotein is targeted to mitochondria by heregulin-induced activation of c-src and the molecular chaperone hsp90. Oncogene 25: 20-31, 2006.
16. Yin L and Kufe D: Human MUC1 carcinoma antigen regulates intracellular oxidant levels and the apoptotic response to oxidative stress. J Biol Chem 278: 35458-35464, 2003.
17. Yin L, Huang L and Kufe D: MUC1 oncoprotein activates the Foxo3a transcription factor in a survival response to oxidative stress. J Biol Chem 279: 45721-45727, 2004.
18. Yin L, Kharbanda S and Kufe D: Mucin 1 oncoprotein blocks hypoxia-inducible factor 1 alpha activation in a survival response to hypoxia. J Biol Chem 282: 257-266, 2007.
19. Yin L, Kharbanda S and Kufe D: MUC1 oncoprotein promotes autophagy in a survival response to glucose deprivation. Int J Oncol 34: 1691-1699, 2009.
20. Agata N, Kawano T, Ahmad R, Raina D, Kharbanda S and Kufe D: MUC1 oncoprotein blocks death receptor-mediated apoptosis by inhibiting recruitment of caspase-8. Cancer Res 68: 6136-6144, 2008.
21. Bartel DP: MicroRNAs: target recognition and regulatory functions. Cell 136: 215-233, 2009.
22. Rigoutsos I: New tricks for animal microRNAs: targeting of amino acid coding regions at conserved and non-conserved sites. Cancer Res 69: 3245-3248, 2009.
23. Filipowicz W, Bhattacharyya SN and Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nat Rev Genet 9: 102-114, 2008.
24. Visone R and Croce CM: MiRNAs and cancer. Am J Pathol 174: 1131-1138, 2009.
25. Lynam-Lennon N, Maher SG and Reynolds JV: The roles of microRNA in cancer and apoptosis. Biol Rev Camb Philos Soc 84: 55-71, 2009.
26. Calin GA, Dumitru CD, Shimizu M, *et al*: Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 99: 15524-15529, 2002.
27. He L, He X, Lim LP, *et al*: A microRNA component of the p53 tumour suppressor network. Nature 447: 1130-1134, 2007.
28. Hermeking H: P53 enters the microRNA world. Cancer Cell 12: 414-418, 2007.
29. Le MT, Teh C, Shyh-Chang N, *et al*: MicroRNA-125b is a novel negative regulator of p53. Genes Dev 23: 862-876, 2009.
30. Lewis BP, Burge CB and Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15-20, 2005.
31. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP and Bartel DP: MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell 27: 91-105, 2007.
32. Friedman RC, Farh KK, Burge CB and Bartel DP: Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19: 92-105, 2009.
33. John B, Enright AJ, Aravin A, Tuschl T, Sander C and Marks DS: Human microRNA targets. PLoS Biol 2: e363, 2004.
34. Duraisamy S, Kufe T, Ramasamy S and Kufe D: Evolution of the human MUC1 oncoprotein. Int J Oncol 31: 671-677, 2007.
35. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A and Enright AJ: MiRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 34: D140-D144, 2006.

36. Gaemers IC, Vos HL, Volders HH, van der Valk SW and Hilkens J: A stat-responsive element in the promoter of the episialin/MUC1 gene is involved in its overexpression in carcinoma cells. J Biol Chem 276: 6191-6199, 2001.
37. Sachdeva M and Mo YY: MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. Cancer Res 70: 378-387, 2010.
38. Rajabi H, Jin C, Ahmad R, McClary C and Kufe D: Mucin 1 oncoprotein expression is suppressed by the miR-125b oncomir. Genes & Cancer 1: 62-65, 2010.
39. Cimmino A, Calin GA, Fabbri M, *et al*: MiR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA 102: 13944-13949, 2005.
40. Orrenius S, Gogvadze V and Zhivotovsky B: Mitochondrial oxidative stress: Implications for cell death. Annu Rev Pharmacol Toxicol 47: 143-183, 2007.
41. Kroemer G, Galluzzi L and Brenner C: Mitochondrial membrane permeabilization in cell death. Physiol Rev 87: 99-163, 2007.
42. Calin GA, Ferracin M, Cimmino A, *et al*: A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 353: 1793-1801, 2005.
43. Mott JL, Kobayashi S, Bronk SF and Gores GJ: MiR-29 regulates Mcl-1 protein expression and apoptosis. Oncogene 26: 6133-6140, 2007.