Coordinated Regulation of Cell Cycle Transcripts by p53-Inducible microRNAs, miR-192 and miR-215

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Abstract

Cell cycle arrest in response to DNA damage is an important antitumorigenic mechanism. MicroRNAs (miRNAs) were recently shown to play key regulatory roles in cell cycle progression. For example, miR-34a is induced in response to p53 activation and mediates G1 arrest by down-regulating multiple cell cycle–related transcripts. Here we show that genotoxic stress promotes the p53-dependent up-regulation of the homologous miRNAs miR-192 and miR-215. Like miR-34a, activation of miR-192/215 induces cell cycle arrest, suggesting that multiple miRNA families operate in the p53 network. Furthermore, we define a downstream gene expression signature for miR-192/215 expression, which includes a number of transcripts that regulate G1 and G2 checkpoints. Of these transcripts, 18 transcripts are direct targets of miR-192/215, and the observed cell cycle arrest likely results from a cooperative effect among the modulations of these genes by the miRNAs. Our results showing a role for miR-192/215 in cell proliferation combined with recent observations that these miRNAs are underexpressed in primary cancers support the idea that miR-192 and miR-215 function as tumor suppressors. [Cancer Res 2008;68(24):10105–12]

Introduction

Events leading to the development of cancer from normal tissue have been well charted, and a necessary step in this process is the dysregulation of cell cycle progression that facilitates the propagation and accumulation of genetic mutations. Within each cell, elaborate machinery exists to halt cell cycle progression in response to various stimuli, including DNA damage. Such regulation allows for DNA repair before its replication and cell division, hence preserving the integrity of the genome. Multiple pathways lead to cell cycle arrest; however, the p53 tumor suppressor pathway has been extensively dissected, and it has been shown that p53 activation leads to both G1 and G2-M arrest (1). Yet, the precise mechanism by which DNA damage leads to cell cycle arrest remains only partially understood.

MicroRNAs (miRNAs) are abundant ~21-nucleotide noncoding RNAs that regulate the stability or translation of hundreds of mRNA targets in a sequence-specific manner. In doing so, miRNAs regulate critical biological processes including cell growth, differentiation, and death (2). Recently, we have gained new insight into cell cycle regulation by identifying transcripts targeted by miRNAs (3–5). Whereas certain miRNAs exert their cell cycle effect through targeting key transcripts, other miRNAs do so through cooperatively down-regulating the expression of multiple cell cycle–related transcripts (5–7). In addition to their effects on the cell cycle, these miRNAs and their family members are aberrantly expressed in human cancers, suggesting a possible role in tumor suppression (7–11).

It has long been observed that p53 activation leads to both induction and repression of transcripts (12). Compared with its well-studied transcriptional activation function, the p53 transcriptional repression function remains relatively uncharacterized. p53 can suppress gene expression via several potential mechanisms, including inhibition of activators, recruitment of corepressors to target promoters, and direct inhibition of the basal transcriptional machinery (13–17). Recently, miR-34a was established as a direct transcriptional target of p53 that contributes to p53 tumor suppressor function through down-regulation of a number of target transcripts (6, 18–23). It is possible that p53-induced miRNAs contribute to the transcriptional repression function of p53. This possibility led us to ask whether miRNAs other than miR-34a might be involved in coordinating the transcriptional and posttranscriptional responses to p53 activation.

In the current study, we show that genotoxic stress promotes p53-dependent up-regulation of miR-192/215. Enforced expression of miR-192 or miR-215 leads to G1 and G2-M cell cycle arrest. Using gene expression profiling and RNAi-mediated gene silencing, we identify a set of downstream effectors of miR-192/215 that includes a number of regulators of DNA synthesis and the G1 and G2 cell cycle checkpoints. By simultaneously regulating the expression of these key cell cycle genes, miR-192/215 may mediate the cell cycle arrest function of p53.

Materials and Methods

Cell culture. HCT116 DICER	extsuperscript{es5} cells were previously described (24). TOV21G and U-2-OS cells were obtained from the American Type Culture Collection. To create the TOV21G p53 matched-pair cell lines, cells were stably infected with lentiviruses encoding either H1-term or p53 shRNA. Requests for reprints: Sara A. Georges, Rosetta Inpharmatics LLC, 401 Terry Avenue North, Seattle, WA 98109.

Gene expression (mRNA and miRNA) analyses. HCT116 DICER	extsuperscript{es5} cells were transfected as described previously, and RNA was isolated at 10 and 24 h following transfection. Microarray analysis was done as previously described (7). Briefly, gene expression data analysis was done with the Rosetta Resolver gene expression analysis software (version 7.1 Rosetta Biosoftware). The down-regulated gene set was annotated by the Gene Ontology database. The levels of miRNA expression were determined by RT-TaqMan analysis as previously described (25).
Cell cycle analysis. HCT116 DICER<sup>ex5</sup> cells were transfected with miRNAs (at 10 nmol/L final concentration) or siRNAs (at 100 nmol/L final concentration) using Lipofectamine RNAiMax (Invitrogen) per manufacturer’s instructions. At 48 h posttransfection, cells were left untreated or treated with nocodazole (100 ng/mL; Sigma-Aldrich) or aphidicolin (2 μg/mL; Sigma-Aldrich) for an additional 18 h. Cells were trypsinized and then collected, fixed, and stained with a propidium iodide solution. Bromodeoxyuridine (BrdUrd) labeling was done per manufacturer’s (BD Pharmingen) instructions. For phospho-histone H3 analysis, cells were fixed and permeabilized with IFP buffer [100 mmol/L PIPES (pH 6.8), 10 mmol/L EDTA, 1 mmol/L MgCl<sub>2</sub>, 0.2% Triton X-100, 4% formaldehyde] and stained with propidium iodide and anti–phospho-histone H3 antibody conjugated to Alexa 488 (Cell Signaling Technology). Cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson) and FlowJo software (Tree Star, Inc).

 Luciferase reporter assay. Luciferase reporter plasmids containing 3’ untranslated regions (UTR) of the miR-192 target genes were obtained from SwitchGear Genomics. The sequence coordinates for each of the SwitchGear reporters used in this study are listed in Supplementary Table 2. Cells were transfected first with miRNAs or siRNAs, as described above, and subsequently transfected 4 to 6 h later with SwitchGear reporter plasmids. A renilla luciferase expression plasmid from dual luciferase system (Promega) was used as an internal control.

 Quantitative reverse transcription-PCR and immunoblotting. Transcript abundance was measured by TaqMan gene expression assay (Applied Biosystems) using hGUS as internal control. Levels of transcripts were quantified using an ABI Prism 7900HT sequence detection system. For immunoblotting, 30 μg of whole-cell lysate extracted in a modified radioimmunoprecipitation assay buffer [150 mmol/L NaCl, 50 mmol/L Tris (pH 7.4), 1 mmol/L EDTA, 1% NP40, 0.1% SDS] were used per sample, and proteins were detected with anti-CDC7 (sc-56275, Santa Cruz Biotechnology, Inc.), anti-LMN2B (MAB3536, Millipore), anti-MAD2L1 (ab55452, Abcam), or anti-CUL5 (Invitrogen). Protein level of β-actin was shown as a loading control (Abcam).

 Network analysis. Networks were constructed using interactions between G<sub>1</sub>-S and G<sub>2</sub>-M checkpoint genes defined in the Ingenuity Pathways Analysis database (Ingenuity Systems) and the miR-192 repression signature. The edges were derived from protein-protein interactions defined in the following databases: BIND (26), BioGRID (27), DIP (28), HPRTD (29), MINT (30), NetPro, Proteome (BioBase)<sup>2</sup>, Reactome (31), Ingenuity, and GeneGo MetaBase (GeneGo).<sup>3</sup>

Results

**miR-192 and miR-215 are up-regulated in response to genotoxic stress.** miR-34a is strongly induced by p53 activation from genotoxic stress (6). To characterize other miRNAs that may be similarly regulated, we measured miRNA expression in two p53 matched-pair cell lines following treatment with the DNA damaging agent doxorubicin. Similar to miR-34a, we found that doxorubicin induced the up-regulation of miR-192/215 in a dose-dependent manner in wild-type, but not p53-deficient, cells (Fig. 1A). The knockdown of p53 in these cells was shown by lack of p21 induction in response to doxorubicin (Fig. 1B). From these observations as well as from the results of Braun and colleagues, (32) we conclude that miR-192 and miR-215 are induced by p53 activity. To delineate the downstream effect of miR-192/215 up-regulation, we investigated the function of miR-192/215 through gene expression profiling.

**miR-192 and miR-215 generate a gene expression signature that is highly enriched for regulators of cell cycle progression.** miRNAs down-regulate gene expression by inhibiting translation of their target transcripts and/or mediating the degradation of these transcripts. Expression profiling has been used to characterize the functions of miRNAs by identifying their targets (7, 33, 34). We introduced synthetic duplexes of miR-192 and miR-215 into HCT116 DICER<sup>ex5</sup>, a human colorectal cancer cell line with hypomorphic DICER function (24). At 10 and 24 hours posttransfection, total RNA was extracted and subjected to gene expression profiling with the Agilent 44k microarray. To study the proximal primary effect of miR-192 and miR-215 expression, we focused our analysis on down-regulated transcripts (Fig. 2A). miR-192 and miR-215 have a common seed region sequence. Consistent with this, they showed virtually indistinguishable
expression profiles (Fig. 2A; Supplementary Table 3). The 3’-UTRs of miR-192/215-regulated transcripts are highly enriched with hexamer sequences complementary to the seed region of miR-192/215 (the E value for hexamer enrichment, or likelihood that hexamer enrichment is due to chance, was determined to be <1e–83). To initially define a biological role for miR-192/215, we queried the gene set described above for enrichment in known members of established biological pathways. Notably, annotation (using the Gene Ontology biological processes) of these miR-192/215-down-regulated transcripts showed enrichment for categories such as “mitotic cell cycle” and “cell cycle,” with high significance (E < 1e–11). This set of sequences (>1,000 sequences) represents both direct miR-192/215 targets and indirect secondary effectors.

To focus on direct targets of miR-192/215, we queried this sequence set for genes that contain miR-192/215 seed-region hexamer complements in their 3’-UTRs. Through such analysis, we identified 62 genes that were down-regulated by miR-192/215 expression as early as 10 hours as well as at 24 hours post-transfection (Fig. 2B; Supplementary Table 4). Annotation of these 62 genes reported the top-ranked category as “cell cycle” with significant expectation (E < 1e–31).

miR-192 induces G1 and G2-M cell cycle arrest. To test the hypothesis that miR-192/215 regulate the cell cycle directly, we examined cell cycle distribution following transfection with miR-192. For this analysis and throughout the rest of this study, we focused on the function of miR-192 because miR-192 and miR-215 are highly homologous and share virtually identical transcriptional profiles (Fig. 2). We introduced wild-type miR-192 and a seed-region mutant synthetic duplex mimetic of miR-192 into HCT116 DICERΔΔ cells by transient transfection and analyzed cell cycle

![Figure 2.](image-url)

**Figure 2.** miR-192 and miR-215 generate a gene expression signature that is enriched for regulators of cell cycle progression. Microarray profiling of miR-192– and miR-215–regulated transcripts. HCT116 DICERΔΔ cells were transfected with luciferase siRNA (Luc siRNA) or miR-192 or miR-215 synthetic duplexes. RNA was isolated at 10 and 24 h following transfection and was compared with RNA isolated from mock-transfected cells. A, a heat map representing those transcripts that are down-regulated in response to miR-192 and miR-215. Color bars represent log2 expression ratios (samples from treated cells / samples from mock-treated cells) of −1.0 (teal) to +1.0 (magenta). This heat map represents a subset of regulated transcripts identified, with enrichment of transcripts containing sequence complementary to the seed region of miR-192/215 (AGGTCA; E value for enrichment <1.0e–83) in their 3’-UTRs, and does not show those transcripts that are unchanged or up-regulated following miR-192/215 treatment. B, a heat map representing transcripts extracted from the overall down-regulated signature, containing transcripts with 3’-UTR matches to miR-192/215 seed region hexamers; transcripts annotated for the Gene Ontology biological process term “mitotic cell cycle” (E value for enrichment, e–31) are indicated.
effects by fluorescence-activated cell sorting (FACS). Compared with mock-transfected (data not shown) or seed-region mutant miR-192 (miR-192mut)–transfected cells, wild-type miR-192–transfected cells showed a substantial decrease in S-phase and an increase in G2-M phase populations (Fig. 3A, top). Similar effects were observed in miR-215–transfected cells (data not shown).

To further investigate miR-192–induced G1 arrest, we treated transfected cells with the microtubule-depolymerizing agent nocodazole (which traps cells at the G2-M phase, and reveals G1 arrest phenotypes; ref. 7). In response to nocodazole, miR-192mut–transfected cells accumulated in the G2-M phase (>90%). In contrast, half of the miR-192–transfected cells accumulated in G1 (Fig. 3A). We also pulse-labeled transfected cells with the thymidine analogue BrdUrd to assay for DNA synthesis defects (Fig. 3B). Such analysis revealed a substantial (BrdUrd positivity: mock, 37.3%; miR-192, 11.9%) reduction in DNA synthesis (Fig. 3B). Together, these results indicate that miR-192 expression prevented cells from completing the G1-S transition.

To address the G2-M arrest phenotype induced by miR-192, we treated transfected cells with the DNA synthesis inhibitor aphidicolin, which causes cells to accumulate in G2 and reveals defects in cell cycle progression through the G2-M transition. As shown in Fig. 3A, when treated with aphidicolin, a substantial fraction of miR-192–transfected cells accumulated in G2-M whereas miR-192mut–transfected cells did not. By measuring the levels of phospho-histone H3 (a mitotic marker) in nocodazole-treated transfected cells, we determined that miR-192 arrests cells in G2 before mitosis (Fig. 3C). Compared with mock-transfected cells, miR-192 caused an 8-fold reduction in phospho-histone H3–positive cells (Fig. 3C). These results, together with the observation that miR-192 down-regulated transcripts are enriched for cell cycle–related genes, suggest that miR-192/215 function to delay cell cycle progression.

Identification of cell cycle targets of miR-192/215. The above results identified miR-192–induced cell cycle arrest phenotypes and specified a set of 62 genes that are down-regulated following miR-192 expression. To identify targets whose modulation may influence the miR-192 phenotype, we silenced these genes individually by siRNA and screened for cell cycle phenotypes that were reminiscent of the miR-192 effect. We transfected HCT116 DICERex5 cells with pools of three different siRNAs per gene and screened for genes whose silencing induced cell cycle arrest at G1 or G2-M. Next, we ranked the siRNA pools tested in our screen according to the percentage of cells arrested in G1 or G2-M. We then ranked the siRNA pools tested in our screen according to the percentage of cells arrested in G1 or G2-M following transfection (as compared with a negative control siRNA). To avoid being misled by possible RNAi off-target effects, we deconvoluted the siRNA pools to identify genes for which at least two different siRNA duplexes from the pool caused the cell cycle arrest phenotype (data not shown). Silencing of many of the 62 genes caused some measure of G1 or G2-M arrest when compared with luciferase siRNA–transfected samples (Supplementary Table 5). We followed up on the 10 genes whose targeting by siRNA caused the largest percentage of cells to arrest in G1 (Fig. 4A) or in G2-M (Fig. 4B). Thus, we compiled a concise list of candidate miR-192 targets that are likely to be involved in the miR-192–mediated G1 and G2-M arrest phenotypes.

To further confirm that these candidate genes are direct downstream targets of miR-192, we used quantitative PCR to measure the transcript levels following miR-192 transfection in another cell line without altered DICER function. For this analysis we used the U-2-OS cell line, an osteosarcoma cell line that has a relatively low endogenous level of miR-192/215. Consistent with our microarray data, we measured a decrease in candidate gene expression.

**Figure 3.** miR-192 causes accumulation of cells at G1 and G2-M cell cycle checkpoints. A, miR-192 triggers the accumulation of cells at the G1 and G2-M phases of the cell cycle. FACS analysis of the cell cycle distribution of HCT116 DICERex5 cells following miR-192 transfection. Where indicated, cells were treated with nocodazole or aphidicolin starting at 48 h posttransfection and were sorted according to DNA content 18 h later (66 h posttransfection). Cell cycle distributions of cells transfected with miR-192mut duplex (which carries a mutation in positions 4 and 5 of the miR-192 hexamer; gray shaded profiles) were compared with miR-192–transfected cells (black line overlays) at 66 h posttransfection. On the Y-axis, the “% of Max” label indicates that the cell cycle distributions of the overlaid samples have been normalized to compensate for potential differences in the number of events in the two samples. B, miR-192 induces a defect in the G1-to-S phase transition. Mock-transfected (left) and miR-192–transfected (right) cells were analyzed for 5-BrdUrd incorporation using flow cytometry. The percentage of cells in S phase in the mock-transfected and miR-192–transfected populations was measured to be 37.3% and 11.9%, respectively. C, miR-192 causes a defect in the G2-M transition. Mock-transfected (left) and miR-192–transfected (right) cells were permeabilized, immunostained for phospho-histone-H3, and sorted for DNA content, and the respective accumulation of positively stained cells in the mitotic compartment was quantified.

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4 M. Dobbelstein, personal communication.
transcript levels in U-2-OS cells as early as 10 hours following miR-192 transfection, relative to transfection with both a luciferase siRNA and the miR-192mut control (Fig. 4C). To test that miR-192 is regulating these genes through seed-sequence–specific recognition of binding sites within their 3′-UTRs, we tested reporter constructs containing the natural 3′-UTRs of the 18 candidate genes inserted downstream of a luciferase open reading frame. We cotransfected these reporters with miR-192 or control RNA duplexes and measured luciferase activity. As shown in Fig. 4D, 3′-UTRs from these 18 genes were regulated by miR-192 but not by miR-192mut, indicating that these 3′-UTRs can confer regulation of a heterologous gene (luciferase) by miR-192. Moreover, miR-192, but not miR-192mut, down-regulated the expression of several of the targets at the protein level (Fig. 4E).

Figure 4. Identification of genes that recapitulate miR-192–induced G1 and G2-M arrest phenotypes. A and B, FACS analysis of the cell cycle distribution of miR-192– or siRNA-transfected cells. HCT116 DICER+ cells were transfected with 10 nmol/L miR-192, 100 nmol/L siRNA against luciferase (Luc siRNA), or 100 nmol/L siRNA against the putative miR-192 target gene of interest, as indicated. At 48 h posttransfection, cells were treated with nocodazole (A) or aphidicolin (B) for an additional 18 h before FACS analysis. A, analysis of G1 accumulation of miR-192–transfected cells versus siRNA-transfected cells. Of the total events analyzed in each sample, the percentage of cells arrested in G1 is shown. B, analysis of G2 accumulation of miR-192–transfected cells versus siRNA-transfected cells. Of the total events analyzed in each sample, the percentage of cells arrested in G2 is shown. siRNAs targeting three genes (CRK, NBN, and TUBGCP3) from the list of 62 genes were shown to have no effect on cell cycle progression. C, miR-192 targets endogenous transcripts. U-2-OS cells were transfected with 10 nmol/L siRNA against luciferase, 10 nmol/L miR-192, or 10 nmol/L miR-192mut. RNA was isolated at 10 h posttransfection and transcript abundance was measured by quantitative PCR. All transcript levels are normalized relative to the abundance of hGUS transcripts; for the graph, the relative abundance of each gene following transfection with luciferase siRNA has been set to 1. D, miR-192 suppresses heterologous 3′-UTR reporter plasmids corresponding to endogenous miR-192 target genes. For each putative miR-192–targeted transcript, the entire 3′-UTR was fused to the luciferase open reading frame to create 3′-UTR luciferase reporter plasmids. U-2-OS cells were transfected with 10 nmol/L miR-192 or miR-192mut at 4 to 6 h posttransfection, cells were cotransfected with 3′-UTR luciferase reporter plasmid and pRL renilla luciferase control. Luciferase activity was measured at 24 h posttransfection and quantified relative to renilla luciferase activity. This graph represents the average normalized luciferase activity as measured in three separate trials conducted in duplicate; for each reporter, the luciferase activity of samples transfected with miR-192mut is set to 1. E, miR-192 suppresses the protein expression of cell cycle genes. HCT116 DICER+ cells were transfected with 10 nmol/L siRNA against luciferase, 10 nmol/L miR-192, or 10 nmol/L miR-192mut, and lysates were prepared at 28 or 48 h posttransfection, as indicated. CDC7, LMNB2, MAD2L1, and CUL5 were detected by Western blot with anti-CDC7, anti-LMNB2, anti-MAD2L1, and anti-CUL5 antibodies, and protein levels were compared with the level of β-actin expression as detected by an anti–β-actin antibody.
miR-192 affects cell cycle progression by coordinated regulation of its targets. As described above, we identified a set of miR-192–regulated genes that, when targeted by siRNA, individually reproduced the miR-192 cell cycle arrest phenotype. Nevertheless, miR-192 down-regulates these transcripts to a lesser degree (typically ~30–40% down-regulation) than the target-specific siRNAs we used (~80% down-regulation). We hypothesized that miR-192–induced cell cycle arrest might arise from the coordinate regulation of this network of downstream targets. If so, a pool of siRNAs targeting the miR-192 downstream transcripts at suboptimal concentrations could phenocopy the cell cycle effects of miR-192. We titrated individual siRNAs targeting each of the 18 candidate genes and found that transfection of the siRNAs at 0.1 to 0.01 nmol/L did not cause G1 or G2-M arrest (Supplementary Fig. 1). We constructed pools consisting of siRNAs (at 0.1–0.01 nmol/L) targeting the G1 and G2-M gene sets and tested whether such pools recapitulated miR-192–induced arrest. As shown in Fig. 5, these pools mimicked the respective miR-192–induced phenotypes, suggesting that silencing of these miR-192 targets can cooperate to elicit the miR-192 phenotypes. Together, these results show that miR-192 regulates cell cycle progression by the direct targeting of a network of cell cycle regulators (Fig. 6).

Discussion

In recent years, many studies have elegantly established the biological roles of miRNAs by showing the ability of a given miRNA to suppress a few key mRNA targets (4, 5, 35). However, miRNAs also influence cellular processes through coordinate regulation of many targets (7, 34). Here we show that miR-192/215 halt cell cycle progression by coordinately targeting transcripts that play critical roles in mediating the G1-S and G2-M checkpoints. Significantly, the regulatory signature of miR-192/215 (Fig. 2) overlaps substantially with canonical G1-S and G2-M cell cycle checkpoint networks (Fig. 6). Consistent with this notion, the enforced expression of miR-192/215 led to arrest in the G1 and G2-M phases of the cell cycle. As direct transcriptional targets of p53, miR-192/215 could contribute to p53-induced arrest by regulating the expression of these key cell cycle transcripts (32).

Gene expression profiling of miR-192/215–expressing cells identified a set of 62 transcripts that contain hexamer sequences complementary to the miR-192/215 seed region in their 3'-UTRs. As expected, individually down-regulating these putative miR-192/215 targets by potent siRNA duplexes resulted in cell cycle arrest. However, the level of suppression of these genes by siRNA exceeded the level of suppression we observed by miR-192...
targeting, and individually administered siRNA concentrations that mimicked the level of miR-192 suppression were inadequate to suppress cell cycle progression (data not shown). Instead, by siRNA pooling experiments we found that simultaneous subtle modulation (<40% decrease of target transcripts) of miR-192 targets phenocopied the miR-192/215 cell cycle effect (Fig. 5). These results suggest that miR-192/215 induce arrest by cooperatively targeting multiple cell cycle transcripts.

Several possible mechanisms might account for the miR-192/215–induced phenotypes reported herein. Among the miR-192/215 targets, there are genes that are essential for the progression of the cell cycle. In addition to regulating cell cycle–related genes directly, miR-192 could also induce arrest through targeting genes that consequently activate the p53-p21 pathway (32). For example, suppression of DTL by miR-192 can promote p53 stabilization as DTL interacts with both the DDB1-CUL4 and MDM2-p53 complexes to destabilize p53 (36, 37). Furthermore, miR-192–mediated suppression of CDC7 might induce p21 (38), providing an additional mechanistic explanation for how miR-192 might function in the p53 pathway. Alternatively, miR-192/215 might target other genes that otherwise act as inhibitors of p53 and/or p21. Together with the results of Braun and colleagues (32), our results suggest that p53 and miR-192/215 act together to coordinate the transcriptional and posttranscriptional events that mediate cell cycle arrest following exposure to genotoxic stress.

With our study, miR-192 and miR-215 join a rapidly expanding group of miRNAs that have been characterized as putative tumor suppressors that regulate cell cycle progression. Consistent with this notion, recent microarray analyses of colon adenocarcinomas found that miR-192/215 expression is significantly reduced in tumor samples relative to matched adjacent noninvolved tissue (32, 39). As the miRNA expression profiles of tumors are increasingly characterized, it will be interesting to learn whether the differential expression of miR-192 will be reported in a broader range of malignancies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 5/20/2008; revised 7/25/2008; accepted 10/20/2008.

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We thank Miho Kibukawa for her superb technical assistance.

Figure 6. miR-192 coordinately regulates multiple members of the canonical G1-S and G2-M cell cycle checkpoint control networks. Shown are the members of the canonical G1-S (A) and G2-M (B) cell cycle checkpoint networks, which were built using Ingenuity Pathways analysis software. The members of these networks that were found to be regulated by miR-192/215 by microarray are shown in blue, whereas the genes that were confirmed as direct miR-192 targets in this study are shown in gold. Green, canonical network genes that are not targeted by miR-192/215. The edges between nodes indicate protein-protein interaction, as defined in the following databases: BIND, BioGRID, DIP, HPRD, MINT, NetPro, Reactome, Ingenuity, and GeneGo MetaBase (see Materials and Methods). In cases where the same protein-protein interaction edge was represented in multiple data sources, we collapsed the edges into a single edge to improve visualization (dotted edges).
References

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