**Mdm2 and Mdm4 Loss Regulates Distinct p53 Activities**

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Abstract

Mutational inactivation of p53 is a hallmark of most human tumors. Loss of p53 function also occurs by overexpression of negative regulators such as MDM2 and MDM4. Deletion of Mdm2 or Mdm4 in mice results in p53-dependent embryo lethality due to constitutive p53 activity. However, Mdm2−/− and Mdm4−/− embryos display divergent phenotypes, suggesting that Mdm2 and Mdm4 exert distinct control over p53. To explore the interaction between Mdm2 and Mdm4 in p53 regulation, we first generated mice and cells that are triple null for p53, Mdm2, and Mdm4. These mice had identical survival curves and tumor spectrum as p53−/− mice, substantiating the principal role of Mdm2 and Mdm4 as negative p53 regulators. We next generated mouse embryo fibroblasts null for p53 with deletions of Mdm2, Mdm4, or both; introduced a retrovirus expressing a temperature-sensitive p53 mutant, p53A135V; and examined p53 stability and activity. In this system, p53 activated distinct target genes, leading to apoptosis in cells lacking Mdm2 and a cell cycle arrest in cells lacking Mdm4. Cells lacking both Mdm2 and Mdm4 had a stable p53 that initiated apoptosis similar to Mdm2-null cells. Additionally, stabilization of p53 in cells lacking Mdm4 with the Mdm4 antagonist nutlin-3 was sufficient to induce a cell death response. These data further differentiate the roles of Mdm2 and Mdm4 in the regulation of p53 activities. (Mol Cancer Res 2008;6(6):947–54)

Introduction

Proper p53 control is critical for its tumor suppressive function. Under homeostatic conditions, p53 is maintained at low levels by the E3 ubiquitin ligase Mdm2, which catalyzes p53 ubiquitination, marking it for degradation by the proteasome (1-3). Mdm2 also binds and inhibits the p53 transcriptional activation domain (4, 5). In response to DNA damage, p53 phosphorylation disrupts Mdm2 binding, leading to p53 stabilization and activation (6). The importance of Mdm2 in p53 regulation was shown in vivo by the lethality of Mdm2−/−/− embryos at 3.5 days postcoitum whereas p53−/−/−Mdm2−/− mice develop without abnormalities (7, 8). Mdm2−/− blastocysts undergo spontaneous apoptosis, and reintroduction of p53 into p53−/−Mdm2−/− mouse embryo fibroblasts (MEF) also induces an apoptotic response (9, 10). Similarly, tissue-specific deletion of Mdm2 in cardiomyocytes, smooth muscle cells, and the central nervous system results in p53-dependent apoptosis (11-14). Thus, Mdm2 regulation of p53 is essential for proper development, and loss of Mdm2 is sufficient to induce p53-dependent apoptosis in many cell types.

A homologue of Mdm2, Mdm4, also binds the p53 transactivation domain, inhibiting its activity (15). However, the role of Mdm4 in p53 protein stability is murky at best. Overexpression of MDM4 inhibits MDM2-mediated degradation of p53 in immortalized human tumor cell lines, suggesting that MDM4 stabilizes p53 (16-19). Likewise, loss of Mdm4 in MEFs containing a p53 mutant that lacks the proline-rich domain yielded lower p53 levels, albeit a more active p53 (20). In contrast, MEFs lacking Mdm4 contain elevated p53 levels (21, 22). Tissue-specific deletion of Mdm4 in the central nervous system is also associated with increased p53 immunostaining (12, 14). Two other studies showed that MDM4 contributes to MDM2 E3 ubiquitin ligase activity (23, 24). Gu et al. (25) have shown that the MDM2-to-MDM4 ratio may affect p53 stability, providing a possible explanation for these seemingly conflicting results. MDM4 protects p53 from degradation when overexpressed at a ratio greater than 2:1 with respect to MDM2. However, when expressed at similar levels, MDM4 inhibits MDM2 autoubiquitination and enhances MDM2 degradation of p53.

Deletion of Mdm2 or Mdm4 in vivo yields cell lethal phenotypes by different mechanisms (7, 8, 21, 22, 26). As indicated, loss of Mdm2 results in apoptosis in all cells examined to date. Examination of embryos from two Mdm4 mutant lines revealed proliferation defects (22, 26). However, the Mdm4 mutants generated by Migliorini et al. (22) also display apoptosis but only in the developing central nervous system. Conditional deletion of Mdm4 specifically in the central nervous system results in a proliferative defect and apoptosis, whereas cells lacking Mdm2 undergo apoptosis (12, 14). Adult cardiomyocytes lacking Mdm4 also die with time (27). These data suggest that Mdm2 and Mdm4 affect p53 activity differently, resulting in distinct p53 functions in different cell types.

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We decided to probe the molecular aspects of this regulation in a different system where we could actually measure p53 half-life. To accomplish this goal, we first asked whether we could generate mice and cells lacking all three genes. Mice lacking p53, Mdm2, and Mdm4 were viable but succumbed to tumorigenesis at a rate similar to that of p53 homozygous null mice. Primary MEFs null for p53 with varying Mdm genotypes were established and infected with a retrovirus encoding a temperature-sensitive p53 mutant. In this system, loss of Mdm2 or Mdm4 led to p53-dependent apoptosis or cell cycle arrest, respectively. In addition, p53 exhibited transcriptional activation of distinct p53 target genes in cells lacking Mdm2 or Mdm4. Analysis of p53 protein half-life revealed that Mdm4 protected p53 from Mdm2-mediated degradation. Understanding the mechanisms by which Mdm2 and Mdm4 regulate p53 is essential to maximize their potential as therapeutic targets for reactivating p53 in tumors.

Results

Mice Lacking p53, Mdm2, and Mdm4 Are Viable

To explore the genetic interaction between Mdm2 and Mdm4 in regulating p53 activity, we attempted to generate mice and cells lacking all three genes. We crossed p53–/–Mdm2–/–Mdm4–/– and p53–/–Mdm2–/–Mdm4–/– mice to generate p53–/–Mdm2–/–Mdm4–/– mice. Triple-null mice were viable and born according to the expected Mendelian ratio (Table 1). Moreover, p53–/–Mdm2–/–Mdm4–/– bred well and were subsequently crossed to generate a cohort of triple-null mice. These mice succumbed to tumorigenesis at a rate similar to that of p53–/– mice. Triple-null and p53-null mice had a mean survival of 135 and 140 days, respectively (P = 0.75; Fig. 1A). Triple-null mice also developed mainly thymic lymphomas (67%) and sarcomas (33%), with a few mice presenting with more than one tumor, similar to p53–/– mice (Fig. 1B; Table 2; refs. 28, 29). Thus, the comparable survival and tumor incidence of p53–/–Mdm2–/–Mdm4–/– and p53–/– mice showed that the principal role of Mdm2 and Mdm4 was proper control of p53 activity.

Loss of Mdm2 or Mdm4 Determines p53 Protein Levels

To analyze the effects of Mdm2 and Mdm4 loss on p53 stability and function, we generated isogenic primary MEFs. Because Mdm2–/– and Mdm4–/– embryos are not viable, we isolated fibroblasts from p53–/–Mdm2–/–, p53–/–Mdm4–/–, and p53–/– embryos and infected them with a retrovirus encoding a temperature-sensitive p53 mutant, p53A135V. The cells were maintained and characterized as populations and are hereafter referred to as TSΔ2, TSΔ4, and TS cells, respectively. The p53A135V allele resembles a p53-null allele in vivo (30). In cells, ~80% of p53A135V resides in the cytoplasm in a

Table 1. Generation of p53–/–Mdm2–/–Mdm4–/– Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Expected</th>
<th>p53–/–Mdm2–/–Mdm4–/– × p53–/–Mdm2–/–Mdm4–/–</th>
<th>Observed (P = 0.78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>6</td>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>7</td>
</tr>
<tr>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>3</td>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>3</td>
</tr>
<tr>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>3</td>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Expected</th>
<th>p53–/–Mdm2–/–Mdm4–/– × p53–/–Mdm2–/–Mdm4–/–</th>
<th>Observed (P = 0.71)</th>
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<tbody>
<tr>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>3.5</td>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>4</td>
</tr>
<tr>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>3.5</td>
<td>p53–/–Mdm2–/–Mdm4–/–</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE: Two different crosses were set up to examine the combined effects of Mdm2, Mdm4, and p53 loss on survival. All possible genotypes of progeny are shown. P values were determined by χ2 test.
Mdm2 and Mdm4 Regulate Distinct p53 Functions

TABLE 2. Tumor Spectrum of p53−/−Mdm2−/−Mdm4−/− Mice

<table>
<thead>
<tr>
<th>Tumor Types</th>
<th>Tumor Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma, n (%)</td>
<td>14 of 21 (67)</td>
</tr>
<tr>
<td>Sarcoma, n (%)</td>
<td>7 of 21 (33)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Spindle cell</td>
<td>1</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>1</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Unclassified</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
</tr>
</tbody>
</table>

NOTE: n = 19; two animals developed both a lymphoma and sarcoma.

nonfunctional conformation at 39°C, whereas at 32°C it migrates to the nucleus and exhibits wild-type conformation and function (31,32). This enabled us to approximate Mdm2−/− and Mdm4−/− contexts at the permissive temperature of 32°C.

To confirm comparable infection of cells, we measured p53A135V mRNA expression by real-time reverse transcription-PCR in TS, TSΔ2, and TSΔ4 cells. At the nonpermissive temperature, all three cell populations expressed comparable p53A135V mRNA levels and, thus, similar infection efficiency (Fig. 2A). To determine if p53A135V was localized as expected (31), we carried out indirect immunofluorescent staining for p53. At 39°C, p53A135V was predominantly localized in the cytoplasm of TS, TSΔ2, and TSΔ4 cells and relocated to the nucleus by 6 hours at 32°C (Fig. 2B). p53 was readily detected in TSΔ2 cells with an exposure of 0.5 seconds. However, the levels of p53 were very low in TS and TSΔ4 cells, requiring 2-second exposures. Western blotting confirmed a difference in the steady-state levels of p53A135V protein among cells with different genotypes (Fig. 2C). The level of p53 was 2.6-fold higher in cells lacking Mdm2 than in control cells. Moreover, the level of p53 was distinctly lower in TSΔ4 cells (0.3-fold) than in TS cells. By comparison to endogenous p53 levels in wild-type MEFs, the levels of p53A135V were higher in TS cells (Fig. 2D). Because cell manipulations often lead to activation and stabilization of p53, this system may represent an activated p53. We could not detect endogenous Mdm2 or Mdm4 by Western blot analysis in any of these cell populations.

Mdm4 stabilizes p53 presumably by inhibiting p53-Mdm2 interactions because Mdm2 and Mdm4 bind the same domain of p53 (15-19). However, other reports implicate Mdm4 in the down-regulation of p53 protein levels (22, 33). To address the effect of Mdm4 loss on p53 stability, we measured p53A135V protein half-life in TSΔ4 cells. TS, TSΔ2, and TSΔ4 cells were cultured at 32°C for 6 hours to allow for conversion of p53A135V to its wild-type conformation, treated with cycloheximide, and monitored for p53 levels by Western blotting (Fig. 3). Thus, in the absence of Mdm4, decreased p53A135V stability was due to Mdm2 as deletion of both Mdm2 and Mdm4 resulted in a very stable p53 protein.

Loss of Mdm2 or Mdm4 Determines Distinct p53 Phenotypes

We have previously shown that expression of p53A135V in p53−/−Mdm2−/− MEFs recapitulates the in vivo apoptotic phenotype of Mdm2-null blastocysts (9, 10). Accordingly, we wished to determine if p53A135V expression in TSΔ4 cells could reproduce the proliferation or apoptotic defects seen in Mdm4−/− embryos (9, 22, 26). We therefore analyzed p53

Figure 2. Endogenous Mdm2 and Mdm4 have distinct effects on steady-state p53 levels. A. Relative p53 mRNA levels of the temperature-sensitive mutant p53A135V were determined by real-time reverse transcription-PCR using RNA from TS, TSΔ4, and TSΔ2 cells cultured at the nonpermissive temperature of 39°C and normalized to a Gapdh internal control. SDs were determined from triplicate samples. B. TS, TSΔ4, and TSΔ2 cells were cultured at 39°C or 32°C for 6 h, fixed, and incubated with a p53 primary antibody and a FITC-conjugated secondary antibody (green). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (blue). Exposure times are indicated. C. Immunoblot of p53 from whole-cell lysates prepared from MEFs after culturing at 32°C for 6 h. β-Actin was used as a loading control. Protein levels were determined by densitometric quantification of immunoblots and relative p53 level is shown. D. A comparison of wild-type (wt) p53 protein levels in MEFs to p53A135V protein levels after retroviral infection of p53-null MEFs by Western blot analysis. Equal amounts of total protein were loaded.
an apoptotic response. These data indicated that the dominant phenotype, apoptosis, was due to loss of Mdm2 and was independent of Mdm4.

We next determined the ratio of G1- to S-phase cells at permissive and nonpermissive temperatures. At 39°C, the G1-to-S ratio of TS and TSΔ4 cells was 0.8 whereas that of TSΔ2 cells was 1.5 (Fig. 4C). TSΔ2A4 cells had a G1-to-S ratio of 2.1. Temperature shift to 32°C for 24 hours had a small effect on the number of TS, TSΔ2, TSΔ4, and TSΔ2Δ4 cells in G1. However, a pronounced G1 cell cycle arrest was evident at 48 hours in TSΔ4 and TSΔ2A4 as indicated by their G1-to-S ratios of 3.7 and 3.5, respectively. Similarly, TSΔ2Δ4 cells had a G1-to-S ratio of 4.3. Although TSΔ2 and TSΔ2Δ4 cells exhibited a high G1-to-S ratio at 48 hours at 32°C, this represented a small number of cells because the majority of these cells had already died by apoptosis (Fig. 4A and B). In sum, the predominant phenotype in cells lacking Mdm4 was a G1 cell cycle arrest in response to p53 activation, whereas cells lacking Mdm2, regardless of the Mdm4 genotype, readily underwent apoptosis.

**Loss of Mdm2 or Mdm4 Enhances Transcriptional Activity of Distinct p53 Targets**

Both Mdm2 and Mdm4 bind the p53 amino terminus, inhibiting its transcriptional activity (15, 34). Although loss of Mdm4 rendered p53 unstable, it still caused a p53-dependent cell cycle arrest likely due to enhanced p53 transcriptional activation. To assess p53 transcriptional activity, we measured induction of endogenous p21, Mdm2, cyclin G, Bax, Perp, and Noxa mRNAs by quantitative real-time reverse transcription-PCR. TS, TSΔ2, and TSΔ4 cells were cultured at 39°C or 32°C for 6 and 12 hours and then harvested for analysis. Interestingly, TSΔ4 cells exhibited a 5-fold increase in Mdm2 levels over TS cells by 6 hours at 32°C (Fig. 5A). In addition, p21 was induced 2.5-fold by 12 hours after temperature shift (Fig. 5B). However, there was no induction of cyclin G, Bax, Perp, and Noxa in the absence of Mdm4. The gene expression profiles were different in TSΔ2 cells; these cells expressed Perp 3-fold over control cells at 39°C and 6-fold higher after temperature shift for 6 hours (Fig. 5C). Noxa was also expressed 3-fold higher than in controls at 32°C and p21 mRNA levels were slightly elevated over control cells 12 hours after temperature shift (Fig. 5D). Expression of cycin G and Bax did not change in TSΔ2 cells. These findings indicated that in the absence of Mdm4, p53A135V activated Mdm2 and p21. In contrast, the apoptotic genes Perp and Noxa were preferentially up-regulated in TSΔ2 cells. Our results suggested that Mdm2 and Mdm4 determined initiation of cell cycle arrest or apoptosis by controlling p53 transcriptional activation of distinct target genes.

**Disruption of Mdm2/p53 Interaction Stabilizes p53 and Initiates Apoptosis in the Absence of Mdm4**

Various factors such as the cellular context and p53 protein levels have been shown to influence p53 initiation of a cell cycle arrest or apoptosis (35). Our data suggest that in MEFs, protein levels regulated p53 response. To directly test whether enhanced p53 protein levels were sufficient to induce cell death, we treated TS and TSΔ4 cells with the Mdm2 antagonist nutlin-3 (36). Cells were cultured at 32°C for 4 hours and for an
Discussion

Knowledge of the mechanisms by which p53 chooses to initiate apoptosis or cell cycle arrest is critical to understanding tumor response on reactivation of p53 (37-39). Clearly, context is important in this decision, but we wanted to further these studies to examine the molecular aspects of this decision.

To examine the roles of Mdm2 and Mdm4 in this process, we first recapitulated Mdm2⁻/⁻, Mdm4⁻/⁻, and double-null contexts by expression of a temperature-sensitive p53 protein in primary MEFs. Although various studies have shown that MDM4 overexpression stabilizes p53 in tumor-derived cell lines (16-19, 40), we show that endogenous levels of Mdm4 are sufficient to stabilize p53A135V from Mdm2-mediated degradation because loss of Mdm4 resulted in reduced p53A135V protein half-life. Thus, in these studies, the levels of Mdm4 overwhelm the amount of Mdm2 present in the cell, resulting in p53A135V stabilization. Because Mdm2 and Mdm4 bind the same domain of p53, p53A135V stabilization likely resulted from Mdm4 impeding Mdm2 access to p53A135V (5, 15).

Importantly, various studies have shown that Mdm2-Mdm4 interactions also regulate p53 stability and function. In human cells, Mdm4 (also called Hdmx) enhances Mdm2 (also called Hdm2) ligase activity and p53 degradation (40). On the basis of structural analysis, Kostic et al. (41) proposed that Mdm2 and Mdm4 heterodimerization via their respective RING domains contributes to enhanced Mdm2 activity. DNA damage also redirects the ligase activity of Mdm2 from p53 to itself and Mdm4, facilitating p53 activation (42). Therefore, cellular context becomes important as the ratios of Mdm2, Mdm4, and p53 might favor Mdm4-p53, Mdm2-p53, or Mdm4-Mdm2 complexes, resulting in p53 stabilization or degradation. Whereas we know that Mdm2 is transcriptionally regulated by p53, little is known about the transcriptional regulation of Mdm4 (43). Further studies of the interactions of these proteins under specific conditions as well as identification of modifications conducive to particular interactions are needed to further understand the mechanisms of p53 response.

In our studies, a very unstable p53A135V in the absence of Mdm4 retained some transcriptional activity. In TSΔ4 cells, Mdm2 was robustly and rapidly induced by p53A135V after temperature shift, indicating that the initial response to enhanced p53 activity may be to restore physiologic p53 levels. The cell cycle inhibitor p21 was also activated, and these cells exhibited a G1-S arrest. Although MEFs lacking Mdm4 exhibited enhanced p53 activity, these cells did not undergo apoptosis. Under the same conditions, MEFs lacking Mdm2 expressed a stable p53A135V and readily underwent apoptosis at 32°C. Furthermore, cells lacking both Mdm2 and Mdm4 died by apoptosis, suggesting that Mdm2 loss and p53 stabilization are the critical determinants of apoptosis in this system. This idea was further supported by the observation that stabilization of p53A135V on treatment of TS and TSΔ4 cells with the Mdm2 antagonist nutlin-3a also induced a prompt cell death response. Thus, stabilization of p53A135V was associated with the activation of apoptotic target genes and was sufficient to induce apoptosis. The prediction then is that tumors will undergo apoptosis if p53 levels are high enough.

Remarkably, mice lacking p53, Mdm2, and Mdm4 were viable. Furthermore, loss of all three genes did not exacerbate the tumorigenic effect of p53 loss alone, indicating that the primary in vivo role of endogenous Mdm2 and Mdm4 levels was control of p53 activity. Nevertheless, the distinct effects of Mdm4 on p53 stability, activity, and function underscore its potential as another mechanism for inactivating p53 in human tumors (44).
Materials and Methods

Mice and Cell Culture

Generation of Mdm4<sup>−/−</sup> and Mdm2<sup>−/−</sup> mice was previously described (8, 26). Mice were crossed with C57BL/6 mice for more than five generations until the background was >90% C57BL/6. MEFs were derived from 13.5-d-old embryos and maintained in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics. 293 Eco Pac packaging cells (Clontech) were maintained per manufacturer’s recommendations. Twenty-four hours after plating, packaging cells were transfected with 3 μg of pBabe empty vector or pBabe-p53A135V using Fugene 6 reagent (Roche). For retroviral transduction of MEFs, viral supernatants from 293 cells were applied to early passage (P0-P5) MEFs in the presence of polybrene (4 μg/mL). Infected MEFs were cultured at 39°C for 24 to 48 h to allow for infection, and then media were supplemented with puromycin (2.5 μg/mL) for selection. Confluent puromycin-resistant pools of cells were split into duplicate dishes and either cultured at 32°C or 3°C and harvested for analysis at the indicated times. Infected cells were continuously cultured in the presence of puromycin. For nutlin experiments, 50 μmol/L of nutlin-3a or nutlin-3b (kindly provided by Michael Andreeff, The University of Texas M. D. Anderson Cancer Center, Houston, TX) was used.

Flow Cytometry and Annexin V Assay

MEFs were harvested by trypsinization, washed in PBS, and then fixed with cold 70% ethanol. On the day of analysis, cells were washed with PBS-T (0.1% Triton X-100), incubated with p53 antibody FL-393 (1:100; Santa Cruz Biotechnology) for 30 min at room temperature, and then incubated with FITC-conjugated secondary antibody (1:400) for 1 h. After washing, cells were incubated with 50 μg/mL propidium iodide in PBS-T (1% Triton X-100) and analyzed by flow cytometry on a Coulter Counter EPICS Profile Analyzer. Annexin V assays were done with an Annexin V-FLUOS kit per manufacturer’s recommendations (Roche).

Immunoblotting and Protein Stability

Cells were harvested by scraping in cold PBS and cell pellets were resuspended in 2× SDS loading buffer. Proteins were resolved by electrophoresis on 10% polyacrylamide gels,
transferred onto a nitrocellulose membrane, and blotted with antibodies to p53 (FL-393, Santa Cruz Biotechnology) and β-actin (Sigma). To measure protein stability, cells were cultured in the presence of 30 μg/mL cycloheximide (Sigma) for the indicated times and harvested for analysis. Protein levels were quantified by densitometry with a Storm 880 Phosphoimager (Molecular Dynamics).

Immunofluorescent Labeling

Cells were cultured under indicated conditions on coverslips in six-well plates, then washed with PBS and fixed with methanol/acetone (50:50%) for 3 min at room temperature. After washing with PBS, cells were incubated with p53 antibody (FL-393) for 16 h at 4°C and then incubated with FITC-conjugated secondary antibody for 1 h. Coverslips were then mounted on slides with mounting media containing 4’,6-diamidino-2-phenylindole (Vector Laboratories). Cells were visualized with a Leica Epifluorescence microscope for the indicated exposure times.

Quantitative Real-time Reverse Transcription-PCR

RNA was extracted from MEFs using an RNasey kit (Qiagen). Reverse transcription reactions were done with the First Strand cDNA Synthesis Kit per manufacturer’s recommendation (GE Healthcare). Real-time reverse transcription-PCR was done according to the manufacturer’s specification (Applied Biosystems). The Primer Express program was used to design primer sequences that were confirmed for specificity by PCR. Gene expression in each reaction. expression in each reaction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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Molecule Cancer Research

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