p53 Regulates Cellular Resistance to Complement Lysis through Enhanced Expression of CD59

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Abstract

It has been recently hypothesized that the CD59 gene has two putative p53-responsive elements that may be involved in defense of host cells from damage by the complement system in inflammation. Here we have examined the roles of these putative p53-binding sequences within the CD59 gene in regulation of CD59 expression. We have shown that both of these potential responsive elements bind p53 in vitro. Knocking down expression of p53 using small interfering RNA led to a 6-fold decrease in CD59 protein expression in HeLa cells. We have previously observed a decrease of CD59 in camptothecin-induced apoptotic IMR32 cells, whereas expression was increased in the surviving fraction compared with untreated cells. Here, we have shown that these changes are associated with altered expression levels and acetylation status of p53. We have also shown that acetylation status of p53 regulates CD59 expression on cells exposed to inflammatory cytokines to model inflammation. Our data suggest that p53 and in vivo positive/negative regulators of p53 could be used to modulate susceptibility of tumor cells to complement lysis in chemotherapy. (Cancer Res 2006; 66(4): 2451-8)

Introduction

Complement is a major component of innate immunity. It eliminates invading microorganisms, transformed cells, and molecular aggregates from tissues and biological fluids (1). Activation occurs through three convergent pathways, in which the complement components are activated in sequential proteolytic cleavages and/or binding previously activated components. This results in the release of chemotactic factors and cell-activating anaphylatoxins, deposition of opsonic fragments, and formation of the cytolytic membrane attack complex (MAC; ref. 1). Complement may be activated on tumor cells by antibodies (2), immune complexes (3), as a consequence of apoptosis (4) or through proteolytic processes (5–7). However, the cytolytic activity of complement is not always sufficient as an immunologic surveillance mechanism, particularly against tumors (8, 9).

Both normal and malignant cells are protected by membrane-bound complement regulators (CReg) that either limit the formation of the C3/C5 convertase enzymes or the assembly of the MAC (1, 8–10). The key complement enzymes, C3/C5 convertases, are inhibited by the CReg complement receptor 1 (CR1, CD35), decay-accelerating factor (DAF, CD55), and membrane cofactor protein (MCP, CD46). These molecules prevent assembly and promote decay of the C3/C5 convertases (C3a and DAF) or serve as cofactors for the plasma serine protease factor I, which irreversibly inactivates C4b and C3b (CR1 and CR2). CD59, on the other hand, inhibits formation of MAC on cell surfaces. CReg are broadly expressed on a wide variety of tissues and cells, including epithelial, endothelial, and circulatory cells, and act as physiologic brakes to complement amplification. C1r, C1s, CD55, and CD59 are expressed on a number of solid tumors and associated cell lines, and it is apparent that the level of expression in malignant tissue is often greater than that seen in the normal surrounding tissue (7, 11–13). Consequently, the increased complement resistance conferred on these membrane-bound CReg has been proposed as a mechanism that facilitates survival of the tumor or the metastasizing tumor cell when it enters circulation (8, 14, 15).

Little is known about the mechanisms by which expression levels of CReg are controlled in cells. p53, a broadly distributed tumor suppressor protein, is a regulator of expression of several proteins. p53 binds to double-stranded DNA in regions identical with or homologous to a consensus sequence containing at least two decamers of the type PuPuPuCA/TT/AGPyPyPy, separated by 0 to 13 bp (16). The list of genes that possess these p53-binding sites is rapidly increasing and includes p21/WAF1, MDM2, GADD45, BAX, cyclin G, cyclin D, IGF-BP3, PCDNA, TGF-a, Ras, and p53 itself (17–27). The p53-binding sites may be either in the promoter regions or within introns. p53 has recently been reported to enhance the transcription of a glycosyl-phosphatidylinositol–linked membrane protein GML (28) that is related to apoptosis and participates in the sensitization of malignant cells to chemotherapy and inflammation (29, 30). GML exhibits structural homology with the MAC inhibitor CD59 (31). Recently, potential p53-responsive elements have been identified in the CD59 gene by analyses in silico (32). A possible participation of p53 in the immune response by modulating the levels of CD59 has been hypothesized, based upon the observation that inflammation is associated with high expression levels of p53 (33, 34) and CD59 (35–37).

In the present study, we have examined a potential involvement of the putative p53-binding sequences in the promoter and intron areas of the CD59 gene in regulation of CD59 expression. For the first time, we have shown a role for p53 and specifically acetylated p53 in modulation of CD59 expression in cells that may be important in inflammation and in immune escape of cancer cells.

Materials and Methods

Cells and treatments. Human epitheloid carcinoma (HeLa), hepatoma (Hep3B), neuroblastoma (IMR32), and promyelocytic leukemia (HL60) cell lines were obtained from the European Collection of Animal Cell Cultures (Salisbury, United Kingdom). HeLa and Hep3B were propagated in DMEM containing 10% heat-inactivated FCS, supplemented with glutamine, penicillin, and streptomycin (Invitrogen, Paisley, United Kingdom). IMR32...
were maintained in RPMI 1640 with 5% heat-inactivated FCS, and HL60 cells were grown in Iscove's modified Dulbecco's medium containing 20% heat-inactivated FCS, both supplemented as above.

Cytokines were purchased from PeproTech (London, United Kingdom) and were added to the cell medium of IMR32, Hep3B, and HL60 for 36 hours at the following concentrations: interleukin-8 (IL-8), 25 ng/mL; IFN-γ, 500 units/mL.

To knock down expression of p53, HeLa cells were transfected using human specific p53 small interfering RNA (siRNA; Cell Signaling Technology, Hitchin, United Kingdom) following the supplier's protocol.

**Induction of apoptosis.** IMR32 (1 07 cells) were plated out in 175-cm² flasks and allowed to grow for 24 to 48 hours until 80% to 90% confluence. Induction of apoptosis was achieved by replacing the medium with fresh RPMI 1640 with additives as above and 5% heat-inactivated FCS containing 1 μmol/L camptothecin (Sigma, Gillingham, Dorset, United Kingdom). Apoptotic cells were harvested at 24 or 48 hours after induction by striking the flask gently 10 times and removing the medium containing apoptotic cells. Surviving IMR32 remained adherent and were briefly trypsinized. Analysis by flow cytometry using Annexin V/FITC and propidium iodide showed that 90% to 95% of loose and floating cells were apoptotic, and that 5% to 10% in the adherent population were apoptotic (data not shown). Cells were then spun at 300 x g at 22°C for 3 minutes and washed in serum-free RPMI before analysis.

**Electrophoretic mobility shift assay.** Oligonucleotides matching the two putative p53-binding sites within CD59 gene (CD59.1 and CD59.2; Fig. 1) and their complementary sequences were purchased from Invitrogen (Table 1). As a positive control, we used a sequence that is an exact match of a previously described p53-binding consensus sequence (20). For the negative control, we employed a sequence with complete mismatch to the p53-responsive element. Each oligonucleotide was incubated in PBS with its complementary sequence to obtain double-stranded DNA. All probes were labeled with horseradish peroxidase using the North2South Direct horseradish peroxidase labeling and detection kit (Perbio Science UK Ltd., Cramlington, United Kingdom). DNA probes (80 ng each) were incubated with 100 ng of wild-type p53 protein (BD Biosciences, Cowley, United Kingdom) for 50 minutes at 22°C in binding buffer [20 mmol/L HEPES/NaOH (pH 7.4), 0.1 mmol/L EDTA, 0.1% Triton X-100, 2 mmol/L MgCl2, 5% glycerol], containing 0.1 mg/mL poly(deoxyinosinic-deoxycytidylic acid). Labeled probes alone and those from the incubation with its complementary sequence to obtain double-stranded DNA. All probes were separated in a 2% agarose gel and detected by autoradiography.

**Western blot analysis.** Cell lysates were subjected to SDS-PAGE on 10% gel. Proteins were transferred onto a nitrocellulose membrane and blocked using PBS/0.1% Tween 20/5% nonfat milk for 1 hour at 22°C. Membranes were then incubated with primary antibodies overnight at 4°C in PBS/Tween 20/milk (10 mL), and then washed thrice in PBS/Tween 20 for 10 minutes on a rotamix. Membranes were then incubated with the secondary antibody (biotin anti-mouse-HRPO, Bio-Rad, Hemel Hempstead, United Kingdom) for 1 hour at room temperature and washed thrice in PBS/Tween. Membranes were developed using the enhanced chemiluminescence Western blotting detection system (Perbio Science UK Ltd.) and imaged using Kodak Medical Imaging film. Data were quantified densitometrically ( Quantity One 4.3.0 software, Bio-Rad), and triplicate measurements were evaluated statistically using Student's t test. Primary antibodies used were mouse monoclonal BRIC229 (International Blood Group Reference Laboratory, Bristol, United Kingdom) for detection of CD59; rabbit polyclonal anti-acetyl-p53 (Lys373, Lys382) and mouse monoclonal anti-p53, clone BP53-12 (Upstate, Dundee, United Kingdom) for detection of acetylated and total p53, respectively; rabbit polyclonal anti-phospho-p42 mitogen-activated protein kinase (MAPK; Cell Signaling Technology) for p42 MAPK detection. We used either sheep anti-mouse-HRPO or sheep anti-rabbit-HRPO as secondary antibodies, both purchased from The Binding Site (Birmingham, United Kingdom).

**Flow cytometry.** Cells (4 x 105) were seeded into 12-well plates and, following incubation with cytokines (HeLa and IMR32), or transfection with the p53 siRNA (HeLa), were washed twice with PBS, then disaggregated with flow cytometry buffer [FCB; PBS containing 15 mmol/L EDTA, 1% bovine serum albumin, 15 mmol/L NaNO3 (pH 7.4)] for intracellular staining, cells were permeabilized using 0.1% Triton X-100. Cells were then resuspended at a concentration of 107/mL. Cells (100 μL; 105) were incubated with 5 μg of specific primary antibody: BRIC229, anti-p53, clone BP53-12, or anti-acetyl-p53 (Lys373, Lys382) for 30 minutes on ice, and the unbound antibody was removed by three washes with FCB. The cells were then incubated with 1:100 dilution of the FITC-conjugated secondary antibody, anti-mouse or anti-rabbit immunoglobulins. The Binding Site antibody was used three more times with FCB and analyzed on a BD FACSCalibur, Becton Dickinson, Oxford, United Kingdom). All measurements were made in duplicate, and each experiment was replicated twice. All results were compared and statistically analyzed by Student's t test. P < 0.05 was considered to show statistically significant differences.

**Complement lysis assay.** Normal human serum (NHS), obtained by umbilical vein puncture from healthy volunteers, separated promptly, and stored at −80°C until use, was the source of complement in all experiments. As a negative control for the complement-mediated lysis, we either inactivated complement by heat treatment (15 minutes at 56°C) of NHS (heat-inactivated NHS) or depleted the NHS of C8 (C8-deficient NHS) using a monoclonal affinity column. Apoptotic and surviving IMR32, following camptothecin treatment, were harvested as described above and washed twice in complement fixation diluent (CFD; Oxoed, Basingstoke, United Kingdom). NHS or C8-deficient NHS were diluted as appropriate in CFD and added to 105 cells (final volume 100 μL) and incubated for 1 hour at 37°C, 5% CO2. Following incubation, samples were centrifuged at 300 x g for 3 minutes, the supernatant was removed, and cells were stained with 2 μg/mL propidium iodide in FCB and analyzed on the FL-2 channel of a BD FACSCalibur flow cytometer. In some experiments, untreated and surviving IMR32 cells were preincubated with the CD59 blocking antibody BRIC229.
(20 μg/mL) for 1 hour at 37°C and washed twice with PBS, and then the serum (NHS or C8d-NHS) was added at appropriate dilutions. Percentage of lysis was calculated by the following equation:

\[
\%\text{ lysis} = \frac{\text{ (%}\text{ lysis} \text{ [cells/NHS]} - \text{ %}\text{ lysis} \text{ [cells/C8d – NHS]})}{(100 - \text{ %}\text{ lysis} \text{ [cells/C8d – NHS]})} \times 100
\]

Chromatin immunoprecipitation. IMR32 cells treated with camptothecin for 24 or 48 hours were separated into apoptotic and surviving fractions, each fixed for 10 minutes at room temperature in tissue culture medium containing 1% formaldehyde. Untreated IMR32 cells were processed according to the same protocol as a control. All further steps of this assay have been described previously (38). Chromatin sonication was done to produce DNA fragments in the range of 300 to 1,000 bp (electrophoretically determined in 1.5% agarose). The immunoprecipitation was done with anti-acetyl-p53 (Lys 373, Lys 382) antibody (Upstate). The naked coimmunoprecipitated DNAs were then used as templates (10 ng of DNA/reaction) in quantitative PCR assays (39) for detection of p53-responsive elements. A primer pair (negative/chromatin immunoprecipitation) designed to a sequence within the 

\[
\text{CD59}
\]

gene that does not bind either IL-8 or IFN-γ (Table 2). The same assay was carried out for Hep3B cells treated with p53 was used as a control for the specificity of immunoprecipitation. The real-time PCR results were analyzed using the comparative Ct method (ΔΔCt) as described by the manufacturer. ΔΔCt, validation experiments showed similar amplification efficiencies for all templates used (difference between line slopes for all templates <0.1). At least two independent experiments were done for each mRNA, and Student’s t test was applied to calculate significance in changes of expression pattern.

Results

p53 binds response elements in the CD59 gene and modulates its expression. First, we investigated whether the two putative p53-responsive elements mapped in the CD59 gene (Fig. 1A) were capable of binding p53 protein in vitro. We did

### Table 1. Deoxyoligonucleotides used in EMSA

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD59.1</td>
<td>5′-TGTTTTAAGGAGACATGTTATTTAATCAAAGCTCATCCTGATTATAA-3′</td>
</tr>
<tr>
<td>CD59.1-complementary</td>
<td>5′-CTGGCTATCCAGCTCATCCTGATTATAA-3′</td>
</tr>
<tr>
<td>CD59.2</td>
<td>5′-TAAATCAGGAATGACTTGGTATTTAATCAAAGCTCATCCTGATTATAA-3′</td>
</tr>
<tr>
<td>Positive control</td>
<td>5′-TGTTTTAAGGAGACATGTTATTTAATCAAAGCTCATCCTGATTATAA-3′</td>
</tr>
<tr>
<td>Negative control</td>
<td>5′-TTAAATCAGGAATGACTTGGTATTTAATCAAAGCTCATCCTGATTATAA-3′</td>
</tr>
<tr>
<td>Negative control-complementary</td>
<td>5′-CTGGCTATCCAGCTCATCCTGATTATAA-3′</td>
</tr>
</tbody>
</table>

### Table 2. Primer pairs used for real-time quantitative PCR

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Primers</th>
<th>Primer concentrations (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>5′-GGATCAGCAAGCGATGTTAGTGA-3′ (F) 5′-GGCGACAGTTGGTATGAGA-3′ (R) 5′-CCGGTACGCTTCATGAG-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R)</td>
<td>900/50 300/900</td>
</tr>
<tr>
<td>p53</td>
<td>5′-AGCCTGGCATGCTATGAGA-3′ (F) 5′-GGCGACAGTTGGTATGAGA-3′ (R) 5′-TAAAACACTGCTATGACCAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R)</td>
<td>50/300 300/300</td>
</tr>
<tr>
<td>CD59</td>
<td>5′-GATGTCAGGAATGACTTGGTATTTAATCAAAGCTCATCCTGATTATAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R) 5′-TAAAACACTGCTATGACCAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R)</td>
<td>300/300 300/300</td>
</tr>
<tr>
<td>CD59.1/ChIP</td>
<td>5′-GATGTCAGGAATGACTTGGTATTTAATCAAAGCTCATCCTGATTATAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R) 5′-TAAAACACTGCTATGACCAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R)</td>
<td>300/300 300/300</td>
</tr>
<tr>
<td>CD59.2/ChIP</td>
<td>5′-GATGTCAGGAATGACTTGGTATTTAATCAAAGCTCATCCTGATTATAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R) 5′-TAAAACACTGCTATGACCAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R)</td>
<td>300/300 300/300</td>
</tr>
<tr>
<td>Negative/ChIP</td>
<td>5′-GATGTCAGGAATGACTTGGTATTTAATCAAAGCTCATCCTGATTATAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R) 5′-TAAAACACTGCTATGACCAA-3′ (F) 5′-GGCGGATGCTTATGAGA-3′ (R)</td>
<td>300/300 300/300</td>
</tr>
</tbody>
</table>

Abbreviations: ChIP, chromatin immunoprecipitation; R, reverse; F, forward.
electrophoretic mobility shift assay (EMSA) for these two elements with recombinant wild-type p53. As a positive control, we used an oligonucleotide that exactly matches previously described p53-binding consensus sequence (20). For the negative control, we employed a sequence with complete mismatch to the p53-responsive element. These experiments (Fig. 1B) unambiguously showed that both putative p53-binding sites within the CD59 gene were capable of interacting with p53 in vitro. To address further whether p53 plays a role in regulation of CD59 expression, we transfected HeLa cells with siRNA, which specifically causes degradation of p53 mRNA. We chose this cell line because we found it to express high levels of both p53 and CD59 (Fig. 2) and the p53 siRNA transfection kit we used for this experiment was optimized for HeLa. Thirty-six hours after the transfection, we prepared cellular lysate that we used for Western analysis (Fig. 2A). Densitometric analysis showed that expression of p53 and CD59 was reduced by 5 ± 0.2 (±SD)-fold and 6.5 ± 0.3-fold, respectively. As a control for the siRNA transfection, we carried out detection for p42 MAPK, which is known to be involved in degradation of p53 (40). We did not detect any change in expression of this protein, suggesting that decreased expression of p53 is a result of the p53 siRNA transfection. Decreased expression of CD59 on HeLa cells transfected with p53 siRNA was confirmed by flow cytometry (Fig. 2B). Expression 36 hours after the transfection was reduced 6 ± 0.2-fold compared with untransfected cells.

Acetylation of p53 modulates CD59 expression in tumor cells exposed to camptothecin. Analogues of the apoptosis-inducing chemotherapeutic agent camptothecin have been used as second-line therapy in cancer patients (41, 42), with improved survival time compared with standard therapy. We have shown that treatment of the neuroblastoma cell line IMR32 with camptothecin induces loss of CD59 on the apoptotic cells (43). To investigate whether p53 was involved in these changes, we first treated IMR32 with camptothecin and examined CD59 expression by Western blotting as above. We confirmed the reduced expression of CD59 in the apoptotic fractions by comparison with untreated cells 1.8 ± 0.2-fold at 24 hours and 2.3 ± 0.2-fold at 48 hours (Fig. 3A); however, in the surviving cells, we detected a 3.6 ± 0.3-fold increase of CD59 expression at both 24 and 48 hours compared with controls. To confirm that gene regulation was responsible for the increased CD59 protein expression in surviving camptothecin-treated IMR32 cells, we measured CD59 mRNA levels. In surviving cells, CD59 mRNA was increased around 4-fold compared with untreated or treated apoptotic cells (Fig. 3B). Furthermore, we examined whether the changes in CD59 expression correlated with susceptibility to complement lysis (Fig. 3C). IMR32 spontaneously activated complement without need for sensitizing antibody as previously described (44). Cells surviving camptothecin treatment were more resistant to complement, lysis being reduced to half that in untreated cells at the same complement dose. To confirm that CD59 was responsible for this
p53 Regulates Expression of CD59

Acetylation of p53 plays in the up-regulation of CD59, we carried out experiments each analyzed in duplicate; bars, SD. Compared sets are shown by columns with interrelated Ps for comparison.

A

IMR32 IMR32/24h apoptotic IMR32/24h surviving IMR32/48h apoptotic IMR32/48h surviving p53

Ac-p53

B

IMR32 IMR32/24h apoptotic IMR32/24h surviving IMR32/48h apoptotic IMR32/48h surviving

Figure 4. Expression of p53 and recruitment to the CD59 gene. A, two different antibodies were used for detection of p53: anti-p53, clone BPS3-12 recognizing all forms of p53 and anti–acetyl-p53 (Lys373, Lys382) antibody. Binding of p53 to its responsive elements CD59.1 (black columns) and CD59.2 (striped columns) in control IMR32 cells is set as 1. Columns, mean for two independent chromatin immunoprecipitation experiments each analyzed in duplicate; bars, SD. Compared sets are shown by columns with interrelated Ps for comparison.

To confirm that changes in the acetylation pattern of p53 upon treatment of Hep3B cells affect binding of p53 to its responsive elements in the CD59 gene, we did chromatin immunoprecipitation assay using antibody that is specific for (Lys373, Lys382) antibody (Fig. 4B). Data showed that the acetylated form of p53 recognized by this antibody is recruited to both p53-responsive elements in the CD59 gene in surviving cells. However, in apoptotic fractions, we detected around 3-fold less acetylated p53 molecules bound to these responsive sequences. These data confirm that acetylated p53 plays a key role in the regulation of CD59 expression in tumor cells (Fig. 4). Performing quantitative PCR on immunoprecipitated DNA with a primer pair designed for a sequence within the CD59 gene that does not bind p53 (negative/chromatin immunoprecipitation, Table 2) gave insignificant number of copies compared with that in genomic DNA in the same reaction (1,460-fold less; data not shown), confirming very low background in our chromatin immunoprecipitation experiments.

Increased expression of CD59 in inflammation is dependent on acetylation of p53. It has been hypothesized recently that p53 is involved in the innate response to inflammatory stimuli by up-regulating the expression of CD59 and thus protecting host cells from innate complement lysis (32). To investigate this hypothesis and address possible mechanisms, we first treated IMR32 cells with cytokines specific for acute (IL-8) and chronic (IFN-γ) inflammation (45). However, quantitative PCR data (not shown) did not show any effect of these cytokines on IMR32 expression of p53 and CD59. Therefore, we performed investigations on human hepatoma cell line Hep3B, previously shown to respond to IFN-γ by decreased CD59 expression (46). We carried out similar cytokine treatments as for IMR32 (Fig. 5). Expression of p53 mRNA was increased by 16% with IL-8 treatment and 26% with IFN-γ (Fig. 5A). However, CD59 mRNA expression was up-regulated by IL-8 (26%) but decreased by IFN-γ (76% of expression in untreated Hep3B cells). To address this discrepancy between mRNA expression levels of p53 and CD59 upon cytokine treatment, we studied their protein levels (Fig. 5B). Expression of CD59 protein was markedly decreased in IFN-γ-treated cells (40% of expression in untreated cells) but increased in IL-8-treated cells by 20% compared with untreated cells (Fig. 5B), correlating with the mRNA data. The overall protein expression of p53 did not change with the cytokine treatments. However, when we used antibody specifically detecting (Lys373, Lys382) acetylated p53, we found a significant decrease (14 ± 3.4%) in p53 acetylation when cells were treated with IFN-γ and increase (18 ± 3.1%) upon IL-8 treatment. Changes in the acetylation of p53, therefore, correlated with expression level of CD59 at mRNA and protein levels (Fig. 5). To address whether these small changes in acetylation of p53 were sufficient to account for the large decrease in CD59 protein expression, we carried out similar cytokine treatment of the p53 null HL60 cells (47). We did not detect significant change in CD59 mRNA upon IFN-γ and IL-8 treatment, supporting the essential role for acetylated p53 (Fig. 5A). However, there was a slight but statistically significant decrease (12%) in expression of CD59 protein in HL60 cells incubated with IFN-γ (Fig. 5B). These data suggest that although changes in expression of CD59 mRNA upon IFN-γ treatment are related to the p53 changes only, this cytokine affects translational machinery as well. The effect of inhibition of protein synthesis by IFN-γ has been shown previously (48, 49). Therefore, changes in p53 acetylation pattern and inhibition of mRNA translation result in synergistic down-regulation of expression of CD59 protein upon treatment with IFN-γ.

acetylated p53 (Fig. 6A). Our data showed that acetylated p53 was recruited to both binding sites within the CD59 gene in Hep3B cells treated with IL-8. This recruitment correlated with the increased acetylation of p53 (Fig. 5B). However, when cells were incubated with IFN-γ, acetylation of p53 was decreased (Fig. 5B) and the p53-responsive elements in the CD59 gene bound less p53 (Fig. 6A). To address whether the decreased binding of p53 to the responsive elements in the CD59 gene is due to reduced availability of acetylated p53 molecules, we carried out another chromatin immunoprecipitation with the antibody specific for all forms of acetylated p53 molecules, we carried out another chromatin immunoprecipitation closely mirrored that of acetylated p53 binding of p53 to its responsive elements in the CD59 gene. 

To control specificity of chromatin immunoprecipitation experiments with Hep3B cells, we carried out quantitative PCR analysis of p53 (black columns) and CD59 (white columns) expression following an incubation of Hep3B and HL60 cells with IFN-γ or IL-8. Results from two independent measurements displayed a significant difference between untreated and cytokine treated Hep3B samples. *, P < 0.05; **, P < 0.01. However, no significant difference was observed between HL60 samples. B, flow cytometry analysis of p53 (black columns; Lys373, Lys382) acetylated p53 (white columns) and CD59 (striped columns) following the same treatments as in (A). *, P < 0.05; **, P < 0.001. Columns, mean of four measurements obtained from two separate experiments; bars, SD.

**Figure 5.** Effect of cytokines on p53 and CD59 expression in IMR32 and Hep3B cells. A, quantitative PCR analysis of p53 (black columns) and CD59 (white columns) expression following an incubation of Hep3B and HL60 cells with IFN-γ or IL-8. Results from two independent measurements displayed a significant difference between untreated and cytokine treated Hep3B samples. *, P < 0.05; **, P < 0.01. However, no significant difference was observed between HL60 samples. B, flow cytometry analysis of p53 (black columns; Lys373, Lys382) acetylated p53 (white columns) and CD59 (striped columns) following the same treatments as in (A). *, P < 0.05; **, P < 0.001. Columns, mean of four measurements obtained from two separate experiments; bars, SD.

**Figure 6.** Chromatin immunoprecipitation analysis of recruitment of p53 to the CD59 gene in living Hep3B cells. Cells were treated with IFN-γ or IL-8 for 36 hours. Immunoprecipitation was carried out either with anti-acetyl-p53 (Lys373, Lys382; A) or anti-p53, clone BP53-12 (B) antibodies. Binding of p53 to its responsive elements CD59.1 (black columns) and CD59.2 (white columns) in untreated cells is set as 1. Columns, mean for two independent chromatin immunoprecipitation experiments each analyzed in duplicate; bars, SD. Compared sets are shown by columns with interrelated P values for comparison.

**Discussion**

The CD59 gene consists of four exons (50), a 5′-flanking region containing the gene promoter and an enhancer located in intron 1 (nucleotides −1155 to −888 upstream of exon 2; ref. 51). The gene contains two putative p53-responsive elements that do not match exactly the p53 consensus binding sequence (ref. 32; Fig. 1A). We first showed by EMSA that these putative p53-responsive motifs are capable of binding p53 in vitro (Fig. 1B), which is in agreement with the recently published prediction that the CD59 gene possesses functional binding sites for the p53 tumor suppressor protein (52). This prediction was based on experiments in vitro with reporter gene constructs (secreted placental alkaline phosphatase) containing the putative p53-responsive sequences from the CD59 gene. In this article, we have further addressed the role of CD59.1 and CD59.2 putative responsive elements within the CD59 gene in living cells (Fig. 2) by knocking down p53 expression by siRNA. Our findings unambiguously showed that p53 is required for high expression of CD59 in HeLa cells, confirming a functional role for these two elements.

Many tumors initially respond to chemotherapy; however, the surviving cancer cells frequently relapse into a multidrug-resistant state (53, 54). Despite the better survival of cancer patients when camptothecin analogues have been used as second-line therapy, our experiments suggested that surviving cancer cells after such treatment have even greater resistance to complement attack (Fig. 3). This is at least in part a result of increased expression of the CReg protein CD59 in surviving cells after the camptothecin treatment. We showed that the expression level of p53 was increased in surviving cells but also to a lesser degree in apoptotic cells that express less CD59 (Figs. 3 and 4). These data did not fit a simple relationship between p53 expression and CD59 levels.

In an attempt to resolve this inconsistency, we examined the role of post-translational modifications of p53 in regulation of CD59 expression. Recently, it was shown that acetylation of p53 may...


Retraction: p53 Regulates Cellular Resistance to Complement Lysis through Enhanced Expression of CD59

The article titled "p53 Regulates Cellular Resistance to Complement Lysis through Enhanced Expression of CD59," which was published in the February 15, 2006, issue of Cancer Research (1), is being retracted at the request of the authors following the release of the conclusions of an internal investigation panel established by Cardiff University to examine allegations of research misconduct in the preparation of the manuscript. The panel found evidence of splicing or pasting affecting Figs. 1B and 4A, without indication that this had been done. The panel feels that although these image manipulations cannot be characterized as "fabrication" because there is no reason to doubt the validity of the underlying science in the article, they represent unacceptable practice when submitting a manuscript for publication. Dr. R.M. Donev accepted full responsibility for these actions. The panel concluded that none of the other coauthors of this manuscript knew, or had reason to suspect, that the data presented in the manuscript had been manipulated by Dr. R.M. Donev.

Four of the five authors agreed to this Retraction. Attempts to contact the first author, Rossen M. Donev, were unsuccessful.

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Reference


Published OnlineFirst November 7, 2013.
doi: 10.1158/0008-5472.CAN-13-2935
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