hGTSE-1 Expression Stimulates Cytoplasmic Localization of p53*

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hGTSE-1 (human G2 and S phase-expressed-1) is a cell cycle-regulated protein mainly localized in the cytoplasm and apparently associated with the microtubules. hGTSE-1 is able to down-regulate levels and activity of the p53 tumor suppressor protein: it binds the C-terminal region of p53 and represses its ability to induce apoptosis after DNA damage. Here we report that, after DNA damage, hGTSE-1 becomes stabilized in a p53-independent way and accumulated in the nucleus. Further characterization of hGTSE-1 localization revealed increased nuclear staining in unstressed cells after treatment with the nuclear export inhibitor leptomycin B, or when a nuclear export signal (NES) located in its C-terminal region was mutated. Finally, we provide evidence that hGTSE-1 ectopic expression, in addition to p53 protein levels down-regulation, is able to enhance cytoplasmic localization of p53. Interestingly, NES-mutated hGTSE-1 accumulates in the nucleus, binds p53 but looses its ability to enhance cytoplasmic redistribution of p53 and to regulate p53 protein levels. Similarly, when wild type hGTSE-1 functions on p53 were analyzed in cells lacking Mdm2, it failed in regulating both p53 localization and protein levels, thus indicating that hGTSE-1 requires an intact NES and functional Mdm2 for the regulation of p53. Our results provide new insights into the mechanism of hGTSE-1 function, whereby its characterized nucleo-cytoplasmic shuttling ability is required to regulate p53.

The p53-mediated response pathway is among the most frequent targets of inactivation in human cancers. The tumor suppressor p53 protein is a nuclear transcription factor that transactivates a variety of genes involved in growth arrest and apoptosis (1). Failure of p53 function can be attributed to the following: (a) inactivation of p53 by mutations altering its DNA binding capability (2), (b) deregulation of proteins that control p53 levels or activity (i.e. cellular or viral oncopgenes) (3–5), and (c) nuclear exclusion that interferes with its role as transcription factor (6).

Regulation of WT-p53 protein is based on a strict physiological turnover including ubiquitination and degradation in the cytoplasm. Nuclear-cytoplasmic shuttling of p53 depends on nuclear export signals (NES) and nuclear localization sequences (NLSs) located in the N and C termini of the protein (7). The main regulator of p53 is Mdm2, which interacts with the N-terminal of p53 thus blocking p53 transcriptional activation and promoting its degradation by the ubiquitin-proteasome pathway (8). Nucleo-cytoplasmic shuttling of p53 seems to be essential for its degradation by Mdm2 (9, 10). Moreover, it has been demonstrated that Mdm2 expression is a key factor that efficiently promotes nuclear export of p53 (11, 12). Importantly, leading to p53 activation and stabilization such as DNA damage, which causes phosphorylation of the N-terminal NES (13), or p14ARF expression, which interferes with Mdm2 activity (14), efficiently inhibit p53 nuclear exclusion. On the contrary, proteins like E1B55k (15), Mot-2 (16), Parc (17), or PSF-TFE3 (18) are able to down-regulate or destabilize p53 by cytoplasmic sequestration, thus emphasizing the importance of p53 relocalization as an effective means to control its function.

The C-terminal domain of p53 has been characterized as a key domain involved in the control of p53 localization (19, 20). Three NLSs and one NES are located within amino acids 316 and 384 of p53. It has been proposed that p53 localization is mainly regulated by the NES masking/NES exposition switch (21). Mdm2-driven ubiquitination or certain mutations of the p53 C-terminal region enhance p53 relocalization to the cytoplasm (22, 23).

Mouse GTSE-1 (G2 and S phase-expressed), previously named B99, is a cell cycle-regulated protein that mainly localizes to the microtubules and is induced by WT-p53 upon DNA damage (24, 25). We have recently shown that its human homologue, hGTSE-1 (26), is able to control DNA damage-induced apoptosis by down-regulating p53 function (27). hGTSE-1 binds the C-terminal domain of p53 (355–393) and induces down-regulation of p53 levels and activity. Although hGTSE-1 binding to p53 seems to be required for repressing p53 activity, it is unclear how hGTSE-1 regulates p53 stability.

Here we report that hGTSE-1 protein accumulates after DNA damage through a p53-independent mechanism concomitant with its relocalization from cytoplasm to the nucleus. By studying hGTSE-1 subcellular localization we found that it is a nucleo-cytoplasmic shuttling protein containing an active NES in its C-terminal region. We also demonstrate that in addition to decreasing p53 levels, hGTSE-1 expression promotes p53 localization to the cytoplasm. Finally, we show that shuttling activity of hGTSE-1 as well as Mdm2 are both required for the regulation of p53 localization and levels.

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† The abbreviations used are: NES, nuclear export signal; NLS, nuclear localization signal; ET, etoposide; TS-p53, temperature sensitive-p53; WT, wild type; LMB, Leptomycin B; GFP, green fluorescent protein.

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EXPERIMENTAL PROCEDURES

Cell Lines and Treatments—Cell lines were cultured at 37 °C in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml). D4H cells are Saos-2 cells expressing the V143A TS-p53 mutant form (28). AT6 cells are U2OS cells expressing the V143A TS-p53 mutant form. SK23 cells are SKOV3 cells expressing the murine mutant form (28). AT6 cells are U2OS cells expressing the V143A TS-p53 mutant. T47D cells express mutated p53. MEF p53+/H11002Val-135 TS-p53 mutant (29). MCF7 cells and U2OS cells contain wild type p53 mutant. SK23 cells are SKOV3 cells expressing the murine mutant form (28). AT6 cells are U2OS cells expressing the V143A TS-p53 mutant form.

Western blot analysis of hGTSE-1 in MCF-7 cells (WT-p53), T47D cells (mutant p53), and Saos-2 cells (null for p53) 24 h after γ-irradiation (400 rads). C, time course analysis of hGTSE-1, p53, and p21Waf-1 protein levels after ET treatment to U2OS cells.

Immunoprecipitation and Western Blotting—Cells were harvested in ice-cold Nonidet P-40 buffer containing 50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40, 0.1% sodium orthovanadate, 2 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM EDTA, and 10 μg/ml each of chymostatin, leupeptin, antipain, and pepstatin. After 10 min at 4 °C lysates were clarified by centrifugation and precleared with 20 μl of Protein A-Sepharose CL-4B (Amersham Biosciences). Then antibody prebound to 20 μl of Protein A-Sepharose CL-4B was added and incubated at 4 °C for 4 h. The resin was washed and bound proteins were eluted in SDS-PAGE sample buffer.

RESULTS

DNA Damage Stabilizes and Relocalizes hGTSE-1 into the Nucleus—We have previously demonstrated that WT-p53 induces murine GTSE-1 transcription through an active p53 binding site located in its promoter region. Nevertheless, similarly to other p53 target genes, mouse GTSE-1 is also induced by DNA damage in cells lacking functional p53 (24).

To characterize the p53-dependent regulation of hGTSE-1 protein, we used human cell lines expressing a temperature-sensitive p53 allele (TS-p53), with a mutant conformation at 37 °C and a WT conformation at 32 °C. As shown in Fig. 1, Western blot analysis of hGTSE-1 protein after WT-p53 induction in different TS-p53 cells. The activity of p53 was followed by monitoring the expression of p21Waf-1 (middle panel). B, Western blot analysis of hGTSE-1 in MCF-7 cells (WT-p53), T47D cells (mutant p53), and Saos-2 cells (null for p53) 24 h after γ-irradiation (400 rads).
hGTSE-1 mRNA levels in Northern blot analysis in the same cellular system (data not shown).

We then analyzed hGTSE-1 protein levels in response to DNA-damaging agents such as γ-radiation and ET in cells containing different forms of endogenous p53: MCF-7 (WT p53), T47D cells (mutant p53), and Saos-2 cells (p53 null). As shown in Fig. 1B, hGTSE-1 expression increased after γ-irradiation (400 rads) in all tested cell lines. Similar results were obtained using 100 μM ET (data not shown), suggesting that hGTSE-1 protein levels increase after genotoxic stress independently of p53 status. A time course experiment after ET treatment was performed to study the behavior of endogenous hGTSE-1 protein upon DNA damage in U2OS cells. As shown in Fig. 1C, Western blot analysis revealed that the hGTSE-1 protein increased 15–18 h after treatment with ET. Furthermore, analysis of p53 response in this context showed that hGTSE-1 becomes accumulated with a slower kinetic compared with both p53 and the p53-induced protein p21Waf-1 (32), detected 3 and 6–9 h after ET treatment, respectively, indicating that hGTSE-1 increases in a late phase after DNA damage.

By analyzing hGTSE-1 intracellular localization we observed that, similar to the murine protein, hGTSE-1 is a cytoplasmic protein localized to the microtubules (26). Overexpression of hGTSE-1, however, revealed a small subpopulation of cells that, in addition to cytoplasmic staining, displayed nuclear staining (data not shown). This observation prompted us to analyze whether a change in hGTSE-1 localization could be associated to a DNA damage response. To this purpose, endogenous and ectopically expressed hGTSE-1 localization were analyzed in U2OS cells 24 h after ET treatment. As shown in Fig. 2A, ET treatment evidenced a significant fraction of cells with nuclear-localized hGTSE-1. Scoring hGTSE-1 localization in cells treated with ET, as well as its localization in cells treated with other DNA damaging agents as methyl methanesulfonate (100 μM) or UV irradiation (20 J/m²) (see “Experimental Procedures”), indicated that DNA damage efficiently relocalized hGTSE-1 to the nucleus (Fig. 2B). Finally, the kinetics of hGTSE-1 relocalization were monitored in U2OS cells ectopically expressing hGTSE-1 at different time points after ET treatment. As shown in Fig. 2C, ET treatment strongly enhanced hGTSE-1 relocalization to the nucleus 13–18 h after damage, coincident with the previous information related to the accumulation of the endogenous protein (Fig. 1C). Altogether these observations indicate that hGTSE-1 becomes stabilized and relocalized to the nucleus in response to DNA damage signals.

**FIG. 2.** Changes in hGTSE-1 subcellular localization after DNA damage. A, immunofluorescence images showing the localization of endogenous (1 and 2) and overexpressed hGTSE-1 (3 and 4) in ET-treated or untreated U2OS cells as indicated. Cells were fixed and processed for immunofluorescence analysis to visualize hGTSE-1 localization using a specific polyclonal antibody and a fluorescein isothiocyanate-conjugated secondary antibody. Arrows indicate cells expressing hGTSE-1. B, diagram showing the percentage of U2OS cells with nuclear hGTSE-1 localization before and after the indicated stimuli. C, hGTSE-1 nuclear localization during a time course analysis after ET treatment in U2OS cells transfected with hGTSE-1 expression vector.
hGTSE-1 Is a Nuclear-cytoplasmic Shuttling Protein

The observation that hGTSE-1 is able to move from the cytoplasm to the nucleus, prompted us to study the mechanism by which hGTSE-1 can change its intracellular localization. To this purpose, we analyzed the intracellular distribution of hGTSE-1 or its mouse homologue GTSE-1 in unstressed U2OS cells after treatment with the nuclear export inhibitor leptomycin B (LMB). 18 h after cell transfection with human or mouse GTSE-1, LMB was added for an additional 12 h. As shown in Fig. 3A, inhibition of nuclear export by LMB correlated with hGTSE-1 and GTSE-1 accumulation in the nucleus, indicating that shuttling of these proteins could be driven by NESs.

Previous studies using a set of mouse GTSE-1 deletion mutants suggested that the C-terminal region of the protein was involved in controlling its localization. Analysis of the mouse GTSE-1 amino acid sequence revealed a conserved leucine-rich sequence (Fig. 3B) conforming to the criteria established for NESs, similar to that observed for HIV-1 Rev protein (33). We generated human and mouse GTSE-1 constructs with a double-point mutation within the putative NES (hGTSE-1 NES/H11002 and GTSE-1 NES/H11002, respectively) by changing isoleucine 628 and leucine 630 to alanine in hGTSE-1, and leucines 648 and 650 to alanine in GTSE-1 as indicated in Fig. 3B. hGTSE-1 NES and GTSE-1 NES constructs were transfected in U2OS cells and 24 h later their localizations were assessed by immunofluorescence. As shown in Fig. 3C, both NES mutants displayed an enhanced nuclear localization, thus identifying the presence of an active NES within GTSE-1 proteins. This result is in accordance with the enhanced nuclear localization of hGTSE-1 and GTSE-1 observed after LMB treatment, because such a drug inhibits nuclear export pathways that require the CRM1 receptor for leucine-rich NESs (34–36). Accumulation of GTSE-1 proteins to the nucleus could imply another control at the level of nuclear entry, in addition to the described regulation of nuclear export. However, point mutation of two putative NLSs starting on amino acids 235 and 542, failed to completely abolish nuclear localization of hGTSE-1 (data not shown), indicating that other signals or other factors could be involved in such activity.

hGTSE-1 Overexpression Enhances Cytoplasmic Localization of p53

We have previously reported that hGTSE-1 is able to bind the C-terminal region of p53 and down-regulate its levels and activity (27). Because the C-terminal region of p53 has been shown to be critical in regulating its stability (37) as well as its subcellular localization (21, 23) we studied the effect of hGTSE-1 expression with respect to p53 localization.

To analyze the effect of hGTSE-1 expression on the subcellular localization of p53, we cotransfected hGTSE-1 and GFP-p53 expression vectors in human osteosarcoma cells lacking p53 (MG-63). We chose the GFP-tagged p53 construct because it represents a sensible and broadly used method to detect p53 localization (10, 12, 19) and is poorly sensitive to hGTSE-1-induced degradation (see Fig. 5C). 24 h post-transfection cells were fixed and GFP-p53 localization was analyzed by GFP fluorescence. As shown in Fig. 4A, hGTSE-1 overexpression markedly increased cytoplasmic localization of GFP-p53. To further control this effect we performed the same experiments in p53-knockout mouse embryo fibroblasts (MEF p53−/−), GFP-p53. Importantly, mouse GTSE-1 induced similar effects.
on p53 cellular distribution (Fig. 4B), strengthening the relevance of these proteins on p53 function. To quantify their ability to induce cytoplasmic localization of p53, three independent experiments were performed considering at least 200 cells coexpressing GFP-p53 and hGTSE-1 or GTSE-1 per assay. As shown in Fig. 4, C and D, hGTSE-1 and GTSE-1 expression significantly correlate with cells displaying increased cytoplasmic p53, thus suggesting that GTSE-1 proteins are able to promote cytoplasmic localization of p53. This result was confirmed by nucleo-cytoplasmic cell fractionation followed by Western blot (see Fig. 5, hGTSE-1 WT). When hGTSE-1 and GFP-p53 were transfected in mouse cells, p53 relocalized to the cytoplasm as efficiently as observed in human cells, indicating that the observed effect is not cell- or species-specific (data not shown).

hGTSE-1 Nuclear Export Is Required for Regulating p53 Localization and Stability—To characterize whether the nucleo-cytoplasmic shuttling activity of hGTSE-1 affected the subcellular localization of p53, we analyzed the effect of the hGTSE-1 NES defective derivative on p53 localization. To address this point we transfected hGTSE-1 or its NES mutant together with GFP-p53 in MEF p53−/− similarly as performed on Fig. 4. As indicated in representative images shown in Fig. 5A, hGTSE-1 NES− displayed an impaired ability in enhancing cytoplasmic relocalization of p53. Fig. 5B shows a comparative analysis using WT and NES mutant hGTSE-1 indicating that an intact hGTSE-1 NES is necessary for promoting nuclear export of p53. We then analyzed this mechanism by performing a cell fractionation assay. To this aim, we transfected MEF p53−/− with an empty vector, hGTSE-1, or its NES mutant together with GFP-p53 as shown in Fig. 5B. After 24 h, cells were harvested and fractionated. Cytoplasmic and cytoskeleton proteins (named “C”) and nuclear proteins (named “N”) obtained from the empty vector, hGTSE-1 WT and hGTSE-1 NES− transfectants, were analyzed by Western blot to detect GFP-p53. DO-1 monoclonal antibody was used to detect GFP-p53, whereas specific anti-hGTSE-1 polyclonal antibody (which poorly recognizes endogenous mouse GTSE-1) was used to detect hGTSE-1. Actin and histone H3 were used as markers of C and N fractions, respectively. As indicated in Fig. 5C, GFP-p53 was preferentially detected in the N fraction when cotransfected with an empty vector. However, GFP-p53 was strongly detected in the C fraction when cotransfected with hGTSE-1 WT protein, but weakly when hGTSE-1 NES− was used, thus confirming our data obtained by microscopy analysis. In addition, the increased accumulation of hGTSE-1 NES− in the...
nucleus when compared with the WT protein was biochemically demonstrated in this cell fractionation, supporting our data presented in Fig. 3C. Altogether these data indicate that shuttling activity of hGTSE-1 is required for promoting nuclear export of p53.

The inability of hGTSE-1 NES− to relocalize p53 to the cytoplasm could be attributed to its defective shuttling activity because it can interact with p53 similarly to the WT protein, as demonstrated by coimmunoprecipitation experiments. HA-tagged hGTSE-1 WT or NES− constructs were cotransfected with GFP-p53 in HEK293 cells and immunoprecipitated using anti-HA antibody. As shown in Fig. 5D, hGTSE-1 NES− bound p53 as efficiently as the WT protein. The same results were obtained by transfecting mouse GTSE-1 WT and NES− proteins and GFP-p53 in U2OS cells (data not shown), thus supporting the notion that hGTSE-1 shuttling activity is required to induce p53 nuclear exclusion.

Previous results indicated that overexpression of hGTSE-1 down-regulates p53 protein levels (27). Because p53 degradation is described to occur mainly in the cytoplasm (38), we investigated whether regulation of p53 protein stability via hGTSE-1 expression was linked to enhanced cytoplasmic localization of p53 by analyzing the effect of WT and NES-defective hGTSE-1 proteins on p53 levels. To this purpose, p53 null MG-63 cells were transfected with vectors expressing WT or NES− hGTSE-1 proteins and GFP-p53, together with an empty vector as a transfection control. To perform this experiment, we transfected low levels of untagged WT p53, to appreciate alterations in its stability, because we have observed that the stable GFP-p53 fusion protein does not represent a sensitive assay for hGTSE-1-dependent regulation (see Fig. 5C). 24 h later cell lysates were prepared and p53 protein levels were assessed by Western blotting. As observed in Fig. 5E (left panel), while hGTSE-1 WT reduced p53 levels, its NES mutant had no effect in regulating p53, suggesting that relocalization of p53 to the cytoplasm could be a mechanism by which hGTSE-1 controls p53 levels. Finally, we investigated whether down-regulation of p53 levels by hGTSE-1 required proteasome activity. Experi-
ments were performed by adding the proteasome inhibitor MG132 to cells transfected in parallel to those shown in the left panel of Fig. 5E. Cells were treated with MG132 during the last 8 h before lysis. As shown in Fig. 5E (right panel), inhibition of proteasome abolished p53 down-regulation by WT hGTSE-1, indicating that proteasome activity is required for the control of p53 stability by hGTSE-1.

Functional Mdm2 Is Required for hGTSE-1-dependent p53 Nuclear Export—The obtained results suggest that hGTSE-1 is able to down-regulate p53 protein levels by a mechanism involving p53 cytoplasmic relocalization. Mdm2 is the major regulator of p53 (4, 5) and strongly promotes p53 nuclear exclusion (11, 12). For this reason we next analyzed the requirement of Mdm2 in the hGTSE-1-dependent regulation of p53 subcellular localization.

Our first approach was to analyze the effect of hGTSE-1 in the subcellular localization of GFP-p53 in Mdm2-deficient cells (MEF p53−/−, Mdm2−/−). Cells were transfected with GFP-p53 together with an empty vector or hGTSE-1 WT expression vector. 24 h post-transfection cells were fixed and GFP-p53 localization was analyzed by fluorescence microscopy as described. C, WT-p53 or 22.23-p53 were transfected together with GFP or GFP-hGTSE-1 as indicated in MEF p53−/−. p53 was stained using a specific polyclonal antibody. P53 localization was then determined in cells expressing GFP or GFP-hGTSE-1 by fluorescence microscopy. D, determination of p53 protein levels in MEF p53−/−, Mdm2−/− cotransfected with WT-p53 together with an empty vector or hGTSE-1 WT (WT). DO-1 monoclonal antibody was used to detect p53, GFP was used as transfection control.

**Fig. 6.** Functional Mdm2 is required for hGTSE-1-dependent regulation of p53 localization and stability. A, GFP-p53 localization on MEF p53−/−, Mdm2−/− overexpressing hGTSE-1. The image shows hGTSE-1 (upper panel) and GFP-p53 (lower panel) staining on the same field. B, MEF p53−/−, Mdm2−/− were transfected with hGTSE-1, Mdm2, or their combination as indicated together with GFP-p53 expressing vector. p53 localization was then determined by fluorescence microscopy as described. C, WT-p53 or 22.23-p53 were transfected together with GFP or GFP-hGTSE-1 as indicated in MEF p53−/−. p53 was stained using a specific polyclonal antibody. P53 localization was then determined in cells expressing GFP or GFP-hGTSE-1 by fluorescence microscopy. D, determination of p53 protein levels in MEF p53−/−, Mdm2−/− cotransfected with WT-p53 together with an empty vector or hGTSE-1 WT (WT). DO-1 monoclonal antibody was used to detect p53, GFP was used as transfection control.
Control of p53 by Nucleo-cytoplasmic Shuttling of hGTSE-1

We show that hGTSE-1 actively shuttles from the cytoplasm to the nucleus as evidenced by the nuclear export inhibitor LMB, which caused clear nuclear accumulation of hGTSE-1. Furthermore, mutation of the C-terminal NES (hGTSE-1 NES) enhanced hGTSE-1 nuclear localization.

Shuttling activity of hGTSE-1 therefore emerged as a potentially critical mechanism for controlling p53 localization and stability. In line with this hypothesis, coexpression of hGTSE-1 and p53 (used as GFP-tagged protein) resulted in increased cytoplasmic distribution of p53 when compared with localization of p53 alone. hGTSE-1 is known to interact with p53 in a region mapped between amino acids 355 and 393 (27), where two NLSs are located (7). Mdm2-dependent ubiquitination of lysine residues found within these NLSs results in enhanced cytoplasmic localization of p53 (22), suggesting that modification to this region could lead to p53 nuclear export. In this context, physical interaction of hGTSE-1 with p53 could affect this region, enhancing p53 cytoplasmic localization. Importantly, hGTSE-1 shuttling activity is required to promote p53 localization to the cytoplasm. Our results clearly demonstrate that NES defective hGTSE-1 failed in enhancing p53 distribution to the cytoplasm, although its ability to associate p53 was comparable with WT hGTSE-1. It is therefore conceivable that cytoplasmic redistribution of p53, as enhanced by hGTSE-1, is not merely because of alterations or modifications of the C-terminal domain of p53 caused by this interaction, rather, our data also indicate the requirement of hGTSE-1 shuttling activity. Interestingly, Mdm2, known to efficiently promote p53 nuclear exclusion (11, 12) is also required for the hGTSE-1 effect on p53 localization, because we observed that hGTSE-1 WT shows an impaired ability in relocalizing p53 in Mdm2-deficient cells.

Similarly to that observed for p53 relocalization, an intact NES and functional Mdm2 seem to be required by hGTSE-1 in regulation of p53 protein levels, indicating a relationship between the ability of hGTSE-1 to relocalize p53 to the cytoplasm and to regulate its stability. Moreover, inhibition of proteasome activity prevents p53 protein down-regulation by hGTSE-1 expression, indicating that cytoplasmic localization of p53 could be accompanied by proteasome-dependent degradation. The Mdm2 ring finger domain and its ability to ubiquitinate p53 have been shown to be critical in promoting p53 nuclear export (11, 12) and degradation in the cytoplasm (10, 38). The possibility that hGTSE-1 could promote p53 nuclear export by enhancing Mdm2-dependent ubiquitination of p53 does not seem to be consistent with our data, because no detectable increase in p53 ubiquitination was observed in the in vitro ubiquitination assay (data not shown). We thus favor the hypothesis that hGTSE-1 accelerates the p53 nuclear export process after Mdm2-dependent ubiquitination.

Nuclear accumulated hGTSE-1 looses its ability to regulate both localization and levels of p53, as demonstrated using NES defective hGTSE-1. Therefore, we could hypothesize that nuclear accumulation of hGTSE-1, in response to DNA damage, could be because of the phosphorylation-dependent mechanism-inactivating NES function. Subsequently, in the post-damage recovery phase, reactivation of hGTSE-1 NES activity through dephosphorylation could be required for the efficient regulatory function of hGTSE-1 on p53.

It is conceivable that a similar function of hGTSE-1 takes place in unstimulated cells during the cell cycle, because we have shown that it can control basal p53 levels preferentially during the S and G2 phases (27). Moreover, p53 localization was reported to change during the cell cycle, with increased cytoplasmic distribution during S and G2 (43, 44), coincident with hGTSE-1 highest expression. Together these data could suggest a physiological function of hGTSE-1 in regulating p53 stability and nuclear exclusion of p53 in unstimulated cells, pos-
possibly during specific phases of the cell cycle that require additional and more stringent negative regulators.

Our results indicate that after DNA damage hGTSE-1 is stabilized independently of p53 status, suggesting that in response to damage, hGTSE-1 could play specific functions in parallel to that involving p53 regulation. In fact, the ability of hGTSE-1 to regulate the cell cycle progression is p53 independent (27). Other proteins such as JNK (42), Pin-1 (40), or p38 kinase (45) play a role in p53 signaling although their regulation does not depend on p53, thus suggesting that p53 function is tightly controlled by independent signaling pathways.

We present here a possible mechanism by which hGTSE-1 can regulate p53 after DNA damage, highlighting the relevance of its nucleo-cytoplasmic shuttling activity and functional requirement of Mdm2. hGTSE-1, like its mouse homologue GTSE-1, is a cell cycle-regulated protein that maintains its S- and G2-specific expression even when induced by DNA-damaging agents (25). We therefore propose a role of hGTSE-1 as part of a negative feedback loop involved in the control of DNA damage and during specific phases of the cell cycle that require more effective means to down-regulate p53 activity and levels.

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REFERENCES

1. Ko, L. J., and Prives, C. (1996) Genes Dev. 10, 1554–1572
2. Kern, S. E., Pietenpol, J. A., Thiagalingam, S., Seymour, A., Kinzler, K. W., and Vogelstein, B. (1992) Cell 70, 441–452
3. Lin, H. J., Eviner, V., Prendergast, G. C., and White, E. (1995) Mol. Cell. Biol. 15, 4536–4544
4. Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997) Nature 387, 296–299
5. Collavin, L., Monte, M., Lazarevic, D., Utrera, R., Dragani, T. A., and Schneider, C. (2000) J. Biol. Chem. 275, 817–825
6. Fischer, U., Huber, J., Boelens, W. C., Mattaj, I. W., and Luhrmann, R. (1995) Cell 82, 475–483
7. Lohrum, M. A., Woods, D. B., Ludwig, R. L., Balint, E., and Vousden, K. H. (2000) Mol. Cell. Biol. 20, 8593–8603
8. Utrera, R., Collavin, L., Lazariev, D., Delia, D., and Schneider, C. (1998) EMBO J. 17, 5015–5025
9. Liang, S. H., and Clarke, M. F. (2001) Cell 103, 2012–2017
10. O’Keefe, K., Li, H., and Zhang, Y. (2003) Mol. Cell. Biol. 23, 6396–6405
11. Geyer, R. K., Yu, Z. K., and Maki, C. G. (2000) Nat. Cell Biol. 2, 569–573
12. Boyd, S. D., Tsai, K. Y., and Jacks, T. (2000) Nat. Cell Biol. 2, 563–568
13. Zhang, Y., and Xiong, Y. (2001) Science 292, 1919–1925
14. Zhang, Y., and Xiong, Y. (1999) Mol. Cell. 3, 579–591
15. Freez, V. A., Yaguchi, T., Hasun, M. K., Mitsuji, Y., Reddel, R. R., and Kaul, S. C. (2002) Exp. Cell Res. 274, 246–253
16. Rodrigues, M., Li, M., Puskas, N., Qin, J., and Gu, W. (2003) Cell 112, 39–49
17. Mathur, M., Das, S., and Samuels, H. (2003) Oncogene 22, 5031–5044
18. Liang, S. H., Hong, D., and Clarke, M. F. (1998) J. Biol. Chem. 273, 19817–19821
19. Ostermeyer, A. G., Runko, E., Winkfield, B., Ahn, B., and Moll, U. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15190–15194
20. Stemmler, M. J., Marchenko, N. D., Jimenez, G. S., Moll, U. M., Hope, T. J., and Wahl, G. M. (1999) EMBO J. 18, 1660–1672
21. Hatakenaka, K., and Xiong, Y. (2000) Mol. Cell. Biol. 20, 8533–8546
22. LeCroy, W. A., and Kanaar, R. (2001) Mol. Cell. Biol. 21, 8521–8532
23. Loehr, A. C., and Wahl, G. M. (2003) Nat. Cell Biol. 5, 563–569
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