p53 and c-Jun Functionally Synergize in the Regulation of the DNA Repair Gene hMSH2 in Response to UV*

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The tumor suppressor protein p53 is critical for guarding the genome from incorporation of damaged DNA (Lane, D. P. (1992) Nature 358, 15–16). A relevant stress that activates p53 function is UV light (Noda, A., Toma-Aiba, Y., and Fujiwara, Y. (2000) Oncogene 19, 21–31). Another well known component of the mammalian UV response is the transcription factor c-Jun (Angel, P., and Karin, M. (1981) Biochim. Biophys. Acta 1072, 129–157). We show here that upon UV irradiation p53 activates transcription of the human mismatch repair gene MSH2. Interestingly, this up-regulation critically depends on functional interaction with c-Jun. Hence, the synergistic interaction of a proto-oncogene with a tumor suppressor gene is required for the regulation of the mammalian stress response through activation of expression of MSH2.

Human MSH2 is a well characterized component of the DNA repair system, homologous to the Escherichia coli mutS product (1, 2). It is, so far, the most frequently impaired gene in hereditary nonpolyposis colorectal cancer (HNPCC)1 (3–5). Patients from families that suffer from HNPCC are characterized by an early onset cancer, especially of the colorectum and endometrium. The mechanism of MSH2-induced cancer is via defects in DNA mismatch repair. Mutations in the coding region of the human gene have been shown to be involved directly in microsatellite instability in hereditary nonpolyposis colorectal tumors, and these tumor cells are typically defective in DNA mismatch correction (1, 6, 7). Thus, there is a direct link between HNPCC genes and the genetic instability caused by this DNA repair enzyme. Another contributing factor in HNPCC carcinogenesis is that a second mismatch repair allele has often been lost. This is responsible for the elimination of mispairs and loops of 2–4 bp (8). p53 may also be directly involved in the recognition of DNA damage (15, 16) and DNA repair (17, 18). Activation of p53 seems to be necessary for DNA repair of UV-irradiated cells (17, 19–32). However, at present no direct connection between p53 and expression of a DNA mismatch repair gene in response to DNA damage has been established.

Carcinogens, such as UV irradiation, regulate gene expression through functional interaction of a proto-oncogene with a tumor suppressor gene that the p53 tumor suppressor. After DNA damage, p53 expression is up-regulated (11, 12), and it can either arrest cell cycle progression, allowing DNA repair, or apoptosis is induced (13, 14). p53 may also be directly involved in the recognition of DNA damage (15, 16) and DNA repair (17, 18). Activation of p53 seems to be necessary for DNA repair of UV-irradiated cells (17, 19–32). However, at present no direct connection between p53 and expression of a DNA mismatch repair gene in response to DNA damage has been established.

EXPERIMENTAL PROCEDURES

Cell Culture and UVB Irradiation—SAOS-2 cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, ACC 243) and were cultured in McCoy’s 5A medium supplemented with 15% fetal calf serum and 1% penicillin/streptomycin. The HaCaT cells were grown in Dulbecco’s modiﬁed Eagle’s medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Prior to irradiation, culture medium was replaced with phosphate-buffered saline. Fresh medium was added after irradiation. Depending on the cell line, UVB doses of 50, 75, or 100 J/m² were used. Cell extracts were prepared at different time intervals after UVB irradiation as indicated.
Expression Vector Construction—The β-galactosidase control vector, the pG3 basic vector, and the pG3 control vector were from Promega (Mannheim, Germany). 887 bp of the hMSH2 promoter were amplified by polymerase chain reaction with primers 5'-aagtggcgtgaacatagctga-3' and 5'-tcagctgcaaggcttgaagcc-3'. To the 5'-end of the first primer a KpnI site was added, and to the second primer a HindIII site was added. The polymerase chain reaction product was purified and finally subcloned into the pG3-luciferase vector. To prepare the p53 and c-Jun mutant hMSH2 promoter, we used the Quick Change Mutagenesis Kit from Stratagene (La Jolla, CA). The p53-binding site 5'-aggctagttt-3' was changed to 5'-aggagcgttt-3' in the mutant version of the hMSH2 expression vector, and the AP-1 site 5'-tgaatca-3' was changed to 5'-ggaagca-3' in the mutant version of the hMSH2 vector.

Expression vectors for p53 and c-Jun have been described previously (38). All plasmids were Qia-tip-purified (Qiagen, Hilden, Germany) and dissolved in Tris/EDTA buffer.

Cell Transfection—The transfection reaction was done with “Dospers” liposomal transfection reagent (Roche Molecular Biochemicals) according to the supplier’s recommendations. One or two days before transfection the cells were seeded on 6-well plates to reach 60–80% confluency on the day of transfection. Each transfection experiment was done with 1 μg of the β-galactosidase construct as internal standard and 2 μg of each different hMSH2 construct together with various amounts of p53 or c-jun expression vectors. Values are the mean of at least three independent experiments. The preparation of cell extracts and determination of luciferase activity in a LUMAT LB 9501 (Berthold, Munich, Germany) was performed according to the supplemented recommendation. The activity of β-galactosidase was detected with the chemiluminescent reporter assay Galacto-Light Plus (Tropix, Bedford, MA) according to the supplier’s recommendations. The measured luciferase activity was normalized to the β-galactosidase expression.

Fig. 1. a, structure of the hMSH2 promoter. Comparison of the hMSH2-p53 motif and the p53 consensus sequences shows an overall 90% similarity, and the deviations in the second p53 motif are indicated. b, increase of hMSH2 expression in HaCaT cells after UV irradiation with 100 J/m².

Fig. 2. a, hMSH2 promoter activity in SAOS-2 cells measured 6 h after irradiation. Various amounts of p53 were added as indicated (ng per dish) in a–c. b, increase of the hMSH2 promoter activity in SAOS-2 cells in the presence of various amounts of p53 expression vector measured 12 h after UV irradiation with 50 J/m². c, hMSH2 mutant promoter activity in SAOS-2 cells after addition of various amounts of p53 expression vector; UV irradiation with 50 J/m²; measurements 12 h after irradiation.
RESULTS

Previously, we have identified a p53-binding motif (−447 5′-aggtacttt-3′ and −416 5′-aagtttccttt-3′; Fig. 1a) in the proximal promoter region of the hMSH2 gene (39). Both sequences are high affinity p53-binding sites in vitro (40). To determine the consequences of p53 binding on transcriptional activation of hMSH2, transient cotransfection experiments were performed using luciferase reporter genes whose expression is driven by the wild-type hMSH2 promoter or by a mutated version in which the p53-binding sites were inactivated by point mutations (Fig. 1a). First, we used HaCaT human keratinocytes expressing endogenous wild-type p53 which is able to bind to its corresponding DNA sequence (41) and to activate transcription (42). In non-treated cells, low basal level expression of the wild-type hMSH2 reporter was measured. This expression could not be increased upon cotransfection of an expression vector encoding wild-type p53 (data not shown) suggesting the endogenous p53 is at saturation for reporter activation. Following UV irradiation, a considerable activation of the hMSH2 promoter was seen (Fig. 1b). Maximal levels were reached within 6 h after irradiation. Importantly, UV-induced activation was lost upon mutation of the p53-binding site (data not shown), demonstrating the functional role of p53 for positive regulation of the hMSH2 promoter by UV.

To confirm this assumption in an independent, more rigorous system, we employed human osteosarcoma SAOS-2 cells, which lack the endogenous p53 gene (43). Cells were cotransfected with wild-type or mutant hMSH2 reporters together with increasing amounts of an expression vector encoding the wild-type p53. Addition of up to 200 ng of the p53 expression vector had no effect on the basal expression level of hMSH2 (Fig. 2). However, when the transfected cells were irradiated with UVB light, a dose- and time-dependent increase in hMSH2 promoter activity was obtained. The maximum response was seen 12 h after irradiation at a dose of 50 J/m² (Fig. 2). By using 75 J/m², similar results were obtained except that the maximum response was seen after 24 h (not shown). In line with the data obtained in HaCaT cells, the p53-binding motif mutated version of the hMSH2 promoter was not able to confer UV responsiveness (Fig. 2c). When an irrelevant cytomegalovirus-driven expression vector was used, reporter gene expression was unaffected by UV treatment (data not shown), ruling out the possibility that alterations in reporter gene expression are caused by sequestration of factors that commonly bind to the cytomegalovirus promoter/enhancer unit driving p53 expression and the hMSH2 promoter. These data indicated that hMSH2 is a new target of p53-regulated gene expression.

The lack of UV irradiation-independent hMSH2 expression...
by p53 might be explained by the requirement of a UV-induced post-translational modification of p53 and/or by the need for additional UV responsive factors.

Interestingly, two "classic" AP-1 (c-Jun/c-Fos)-binding sites (44) are present in the hMSH2 promoter flanking the p53-binding motifs (Fig. 1a). This opened the possibility that c-Jun interacts with p53 to regulate hMSH2 promoter activity. To clarify this point, the AP-1-binding site located 5' of the p53-binding motif was mutated (Fig. 1c). In the presence of cotransfected p53 expression vector, no increase in hMSH2 promoter activity was observed even after appropriate UV irradiation (Fig. 3a). Thus, the requirement of AP-1 in hMSH2 regulation is indicated.

To establish the critical role of c-Jun in hMSH2 regulation by an independent assay, we measured UV-dependent hMSH2 activity in the presence of a dominant-negative version of c-Jun lacking the transactivation domain. This mutant (TAM67) is fully capable of competing with endogenous c-Jun for dimer formation with other Jun, Fos, and ATF proteins resulting in transcriptionally inactive complexes (45). In SAOS-2 cells in the presence of TAM67, p53-dependent UV induction of the wild-type hMSH2 promoter was completely abolished (Fig. 3b). In the p53-positive HaCaT cells, expression of TAM67 was sufficient to suppress UV induction of the hMSH2 promoter (Fig. 3c). These data demonstrate that functional c-Jun containing AP-1 complexes, which bind to the hMSH2 promoter, are necessary for UV-dependent regulation of this gene.

By having established a critical role of c-Jun in the regulation of hMSH2 transcription, we wanted to know whether strongly enhanced c-Jun/AP-1 levels were sufficient for induction of the hMSH2 promoter in the absence of p53. Therefore, we cotransfected the hMSH2 promoter reporter gene into SAOS-2 cells with a c-jun expression vector in the absence of a p53 expression vector. Under these conditions, no UV induction was seen (data not shown). However, when both p53 and c-jun were present, hMSH2 induction levels were comparable to those after UV irradiation in the absence of exogenous c-jun (Fig. 4; compare with Fig. 3, a and b, and Fig. 2b). In contrast, the promoter containing mutations in the AP-1 site is not responsive to c-jun and p53 overexpression. This shows that enhanced levels of c-jun can substitute for the UV signal and that UV-dependent p53 up-regulation of the hMSH2 promoter is mediated, most likely, exclusively through c-Jun. However, it is important to note that overexpression of c-jun alone is not sufficient for activation of the wild-type promoter because c-Jun-specific transactivation strictly depends on cotransfection of the p53 expression vector (Fig. 4). These data further underline the requirement for functional synergism between AP-1 and p53 in transcriptional regulation of a DNA repair enzyme in response to UV irradiation.

**DISCUSSION**

Our study demonstrated that activation of p53 by a DNA-damaging stimulus is necessary but not sufficient for transcriptional activation of the human mismatch repair gene MSH2. Rather, induction critically depends on a functional synergism between p53 and the ubiquitous transcription factor c-Jun.

A connection between c-Jun and p53 in the regulation of cell proliferation and cell cycle progression has been defined recently. In the absence of c-Jun, the basal level of p53 is increased resulting in reduced cell proliferation. Vice versa, overexpression of c-jun down-regulated p53 promoter activity, suggesting that c-Jun exhibits its function in cell cycle regulation by repression of p53 (36). At first glance, this type of negative interference appears not to be compatible with the functional synergism between both proteins on hMSH2 regulation. However, it is reasonable to assume that negative interference between c-Jun and p53 may represent a safety device to counteract the harmful consequences of long term overexpression of p53 and c-jun, such as induction of a permanent cell cycle arrest, apoptosis, or cell transformation (46). UV-induced expression of hMSH2, and possibly other factors, will only be favored under conditions where both proteins are present in an appropriate ratio. This interpretation is in line with our findings that UV-dependent induction of the hMSH2 promoter was most efficient using 10 ng of p53 expression vector but was less pronounced with higher amounts of expression vector (Fig. 2b). Similar results were observed with the expression vector encoding c-Jun (data not shown).

The mechanism how hMSH2 acting as a mismatch repair gene is involved in the response mechanism to UV damage is not completely clear. It was shown that the human MutSα mismatch repair protein, a heterodimer composed of hMSH2 and hMSH6, binds specially to mismatched bases opposite to UV light photoproducts (47, 48). It was suggested that the mismatch repair system is an initial step of the damage signaling and repair cascade (49).

The finding that p53 and c-Jun up-regulate the promoter of hMSH2 provides a novel mechanism that functionally links a tumor suppressor and cell cycle regulator directly to DNA damage repair. To our knowledge, no DNA repair enzyme has been identified by functional means whose expression is regulated by c-Jun. Therefore, hMSH2 represents not only a new p53 but also a novel AP-1 target gene thus making the first link between one of the main genotoxic response transcription factors and DNA repair. On the other hand, our results on the role of p53 in hMSH2 regulation are in line with a recent report describing p53 as a transcription activator of a ribonucleotide reductase subunit. This enzyme catalyzes the rate-limiting step for the production of dNTPs, which are not only required for replication but also for DNA repair. The p53-regulated subunit was shown to be critically involved in this process (18). It is tempting to speculate that p53, possibly assisted by c-Jun/AP-1, orchestrates an even broader set of DNA repair functions. In the future, it will be interesting to analyze other DNA repair genes for p53 and/or c-Jun dependence.
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