

PARTICIPATION OF TUMOR SUPPRESSOR P53 IN THE EXPRESSION OF ACUTE-PHASE PROTEIN GENES. Mirjana Mihailović, G. Poznanović, Svetlana Dinić, Aleksandra Uskoković, Nevena Grdović, Melita Vidaković, Jelena Arambašić, Ilijana Grigorov, Svetlana Ivanović-Matić, Vesna Martinić, M. Petrović, and Desanka Bogojević. *Laboratory of Molecular Biology, Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia*

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The AP response is a complex systemic reaction which is activated to restore a homeostatic imbalance after trauma, infection, and mechanical, thermal, or chemical injuries (Bumann and Gauldie, 1994). During the AP response, pro-inflammatory cytokines upregulate transcription factors that, with the major stress-hormones corticosteroids and catecholamines, induce the expression of AP protein genes in the liver. The most prominent AP proteins in the rat are haptoglobin (Hp) and α_2 -macroglobulin (MG). The expression of Hp and MG is regulated at the transcriptional level, relying on the binding affinity of hepatocyte DNA-binding proteins to the respective promoter regions. The p53 tumor-suppressor protein is a nuclear protein (Fields and Janj, 1990; Wang and Privet, 1995) that is latent in normal cells. However, a variety of conditions and agents that cause genotoxic stress rapidly induce its expression. Transcription factor p53 plays a crucial role in the inhibition of cell proliferation (Soussi and May, 1996), modulation of gene transcription (Ginsberg et al., 1991) and induction of apoptosis (Haupt et al., 1995). The ability of p53 to regulate transcription is linked to its affinity for a specific DNA sequence in the promoter element. The p53 consensus DNA-binding sequence is 5'-puPuPuC(A/T)(A/T)GpyPy-Py-3' (Eldery et al., 1992).

Examination of the hormone response element (HRE) (-170/-56) of the Hp gene, located in the promoter proximal region, revealed a potential p53-binding sequence at position -156/-145. Computer search of the extended MG promoter element (-852/+12) revealed the presence of two potential p53 binding sites, at positions -289/-298 and -131/-140 (Algen Promo Computer Gene Analysis, available at: www.algen.Isi.upc.es/cgi/bin/promo). In the present study, we examined the potential role of p53 in transcriptional regulation of the Hp and MG genes in rat liver under basal conditions and during the AP response.

Experiments were performed on 2.5-month-old male albino rats of the Wistar strain. The AP response was induced by a sub-cutaneous injection of turpentine oil (1 μ l/g of body weight) in the lumbar region (Bumann et al., 1984). Soluble nuclear proteins were prepared (Gorski et al., 1986) from livers of control rats and rats sacrificed 12 h after turpentine administration, when maximal transcriptional activity of

the MG gene was observed (Fletcher et al., 1988; Glibetić et al., 1992). Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970), then blotted and examined by immunoblot analysis (Towbin et al., 1979) with p53 antibody (FL-393, Santa Cruz Biotechnology, USA) (Fig. 1A).

Immunoblot analysis revealed the presence of p53 in the control nuclear extract (Fig 1A, lane 1). Induction of the AP response led to slight increases in the relative amounts of p53 (lane 2). Taking into account the existence of a sequence that is homologous to the consensus p53 DNA-binding site in the promoter regions of Hp and MG genes, we used DNA-affinity chromatography (Kadonaga and Tijan, 1986) and Western immunoblot analysis to examine whether p53 possesses binding affinity for the examined promoter sequences under physiological and AP conditions. Equal amounts of nucleoproteins were isolated from control and turpentine-treated rats and

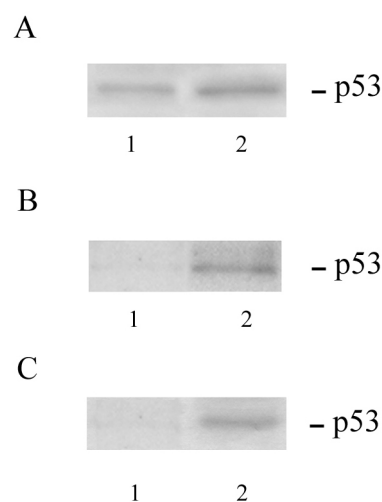


Fig. 1. Western blot analysis of rat liver nuclear extract proteins with p53. A – nuclear extracts; B – nuclear proteins after DNA-affinity chromatography coupled with hormone response element of the haptoglobin gene; C – nuclear proteins after DNA-affinity chromatography coupled with the promoter region of the α_2 -macroglobulin gene. Lane 1 – control; lane 2 –12 h after induction of the AP response.

applied to a DNA affinity column coupled with the Hp gene HRE. After elution with 1 M KCl, the samples were subjected to SDS-PAGE, followed by Western analysis. As can be seen from Figure 1B, p53 displayed a binding affinity for Hp HRE only after induction of the AP reaction (lane 2). The participation of p53 in transcriptional regulation of the MG gene was examined by DNA-affinity chromatography with the extended MG gene promoter, followed by immunoblot analysis (Fig. 1C). It turned out that P53 did not bind to the MG promoter (lane 1) in the control adult liver, whereas 12 h after induction of the AP response it exhibited promoter binding (lane 2). These results point to the AP-dependent binding of p53 to the promoter regions of both of the examined AP proteins.

Basal and AP-induced Hp and MG gene transcription is mostly under the control of liver-enriched C/EBP and STAT families of transcription factors (Ruminy et al., 2001; Bogojević et al., 2003). The results presented here show that p53 also participates in the transcriptional regulation of Hp and MG genes under certain pathological conditions. The turpentine-induced sterile tissue injury used in this study imitates the naturally occurring AP response (Baumann et al., 1984). Inflammation and hypoxia are frequently associated, but their interaction is poorly understood (Yan et al., 1995). The rate of AP protein synthesis is determined at the level of gene expression and is believed to be mainly regulated by cytokines. Published data suggest that genes for interleukins 1 and 6, the mediators of the AP response, are induced by hypoxia (Koj, 1998). Exposure of human hepatoma cells to moderate hypoxia stimulates AP gene expression (Wenger et al., 1995). These findings point to a direct link between low oxygen supply and induction of the AP response. At the same time, hypoxia is a physiological inducer of wild-type p53 (Won et al., 1998). These findings, together with our previous results (Bogojević et al., 1998; 2002; Mihailović et al., 2005) and

the findings presented here, suggest that hypoxia, by inducing p53, promotes its participation in the inducible expression of Hp and MG genes, and most likely of other AP proteins as well.

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