

The Growth Inhibitor TOE1 Interacts with p53 and Modulates Its Transactivation

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Abstract

The TOE1 protein (target of EGR1) has been found to induce a G2/M growth arrest when expressed in a variety of cell types. Our previous work has shown that TOE1 expression correlates with the upregulation of active p21. To better understand the mechanism of TOE1 induced growth arrest, we tested if TOE1 interacts with the p53 tumor suppressor protein and cooperates in the induction of p21. We have used both co-immunoprecipitation and GST pulldown assays to demonstrate an interaction between TOE1 and p53. We define the p53 interaction to include the C terminal portion of the protein representing the tetramerization domain. To quantitate the binding affinity, we have used a label-free method to quantitate biomolecular interactions based on nano-pore optical interferometry. We demonstrate the functional significance of this interaction by determining that the activation of a p53-sensitive promoter is modulated by the interaction between TOE1 and p53. These results provide evidence that TOE1 is able to suppress cellular growth, at least in part, through its binding to and modulation of the activity of p53.

Introduction

The immediate early transcription factor Egr1 is capable of regulating the growth property of many tumor cell types. Published data indicate that one possible molecular mechanism for the biological activity of Egr1 functions through affecting p53 function. Given that Egr1 is a rapid response transcription factor, we consider that identifying its target genes will provide important clues to reveal the mechanism of action of Egr1.

Using chromatin immunoprecipitation we have previously cloned a gene which we named TOE1 (Target Of Egr1). Expression of TOE1 resulted in an accumulation of cells in the G2/M cell cycle phase with a concomitant increase in p21 protein levels. To determine whether TOE1 represents a pathophysiologically relevant Egr1 target gene we explored the possibility that TOE1 may modulate the activity of p53 through protein/protein interactions.

TOE1 affects growth inhibition through increased p21 expression

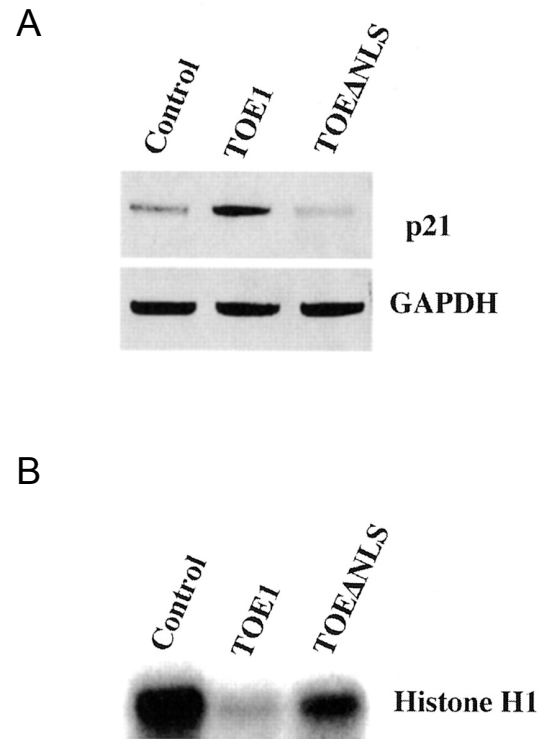


Figure 1

TOE1 expression results in upregulation of active p21 protein levels. A: Western blot for p21/GAPDH
B: in vitro phosphorylation of cyclin B1 immunoprecipitates using a histone H1 substrate.

GST Pull down of TOE1 and p53

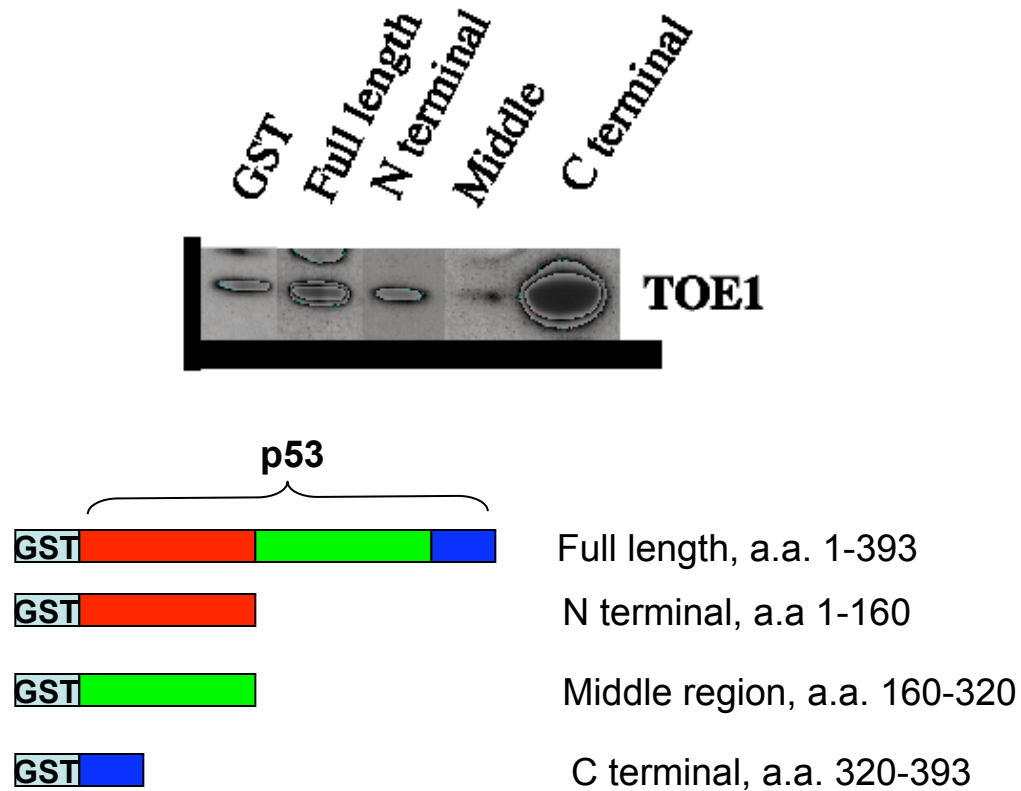


Figure 2

Extracts made from TOE1 expressing cells were incubated with the indicated region of a GST-p53 fusion protein. Following washing and SDS-PAGE, interaction was detected by Western blotting using TOE1 specific antibodies.

* TOE1 interacts preferentially with the C terminal tetramerization domain of p53

Co-Immunoprecipitation of TOE1 with p53

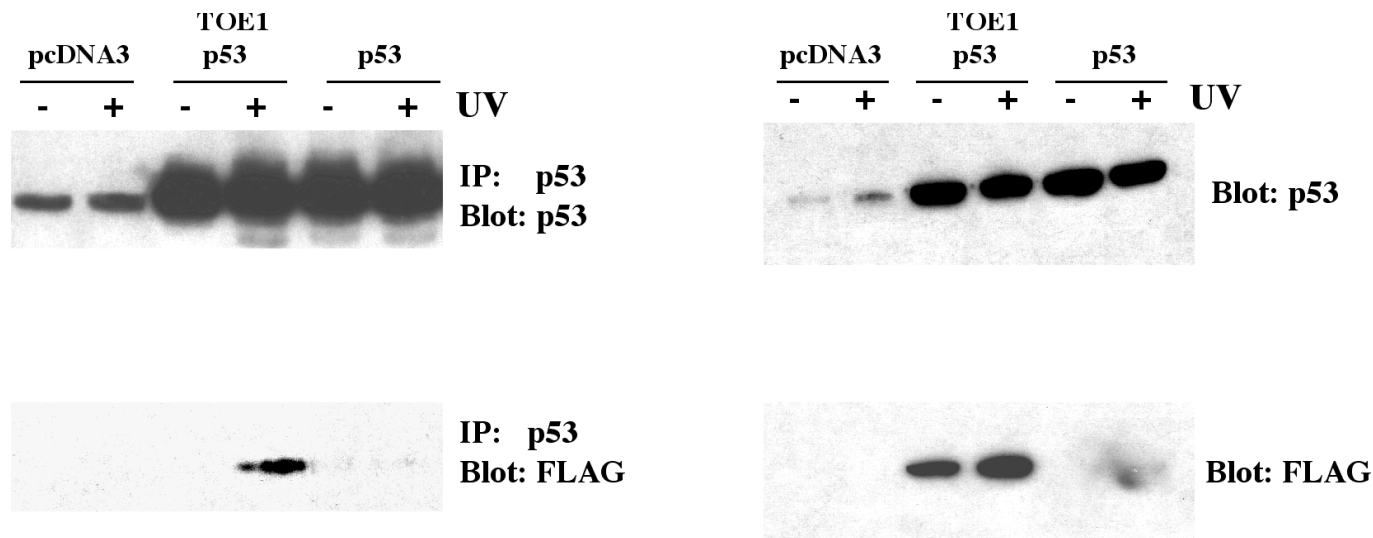


Figure 3

293 cells were transfected with empty vector (pcDNA3), or vectors expressing p53 alone or together with FLAG-tagged TOE1. Following transfection, cells were untreated or exposed to 40J/m² UV. Lysates were immunoprecipitated with rabbit anti-p53 antibodies and blots were probed for p53 or TOE1 with murine antibodies. Left and right panels show immunoprecipitates and whole cell lysates respectively.

* TOE1 binds p53 preferentially following exposure to UV irradiation

TOE1 is a phosphorylated during UV-induced signaling

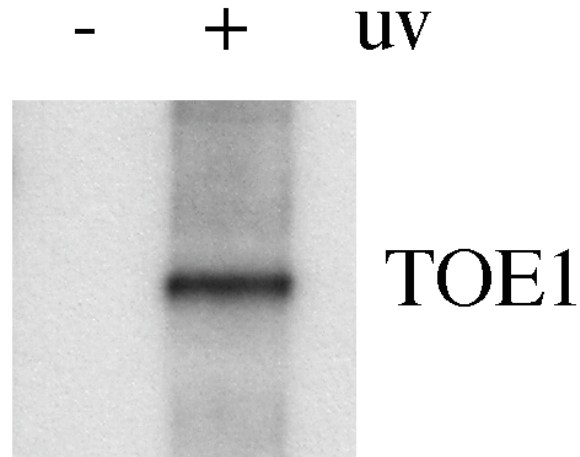
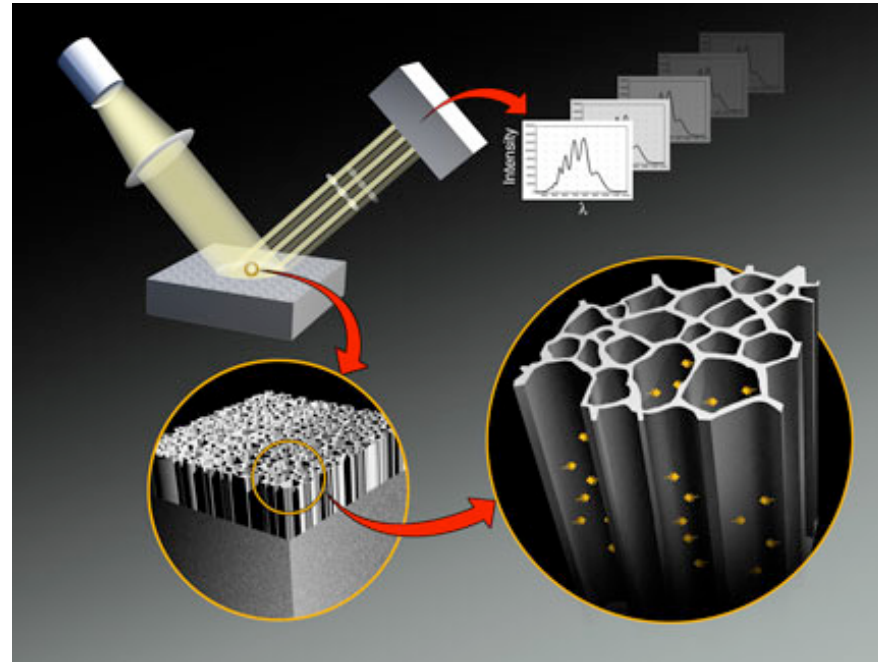


Figure 4

HT1080 cells were transfected with a TOE1 expression vector and untreated or exposed to 40 J/m² uv irradiation. Cells were labeled with ³²P inorganic phosphate for 2 hours and then Extracted and immunoprecipitated with TOE1 antibodies. Phosphorylated TOE1 is seen following uv exposure.

Silicon Kinetics Nanoporous Optical Interferometry



Light reflected off the top silicon surface interferes with light which passes through the porous region, then reflected off the bulk silicon bottom. Biomolecular interactions in the porous region change the index of refraction in that region, creating shifts in the interference pattern, which correlates directly with the rate of biomolecular interaction of interest.

Nanoporous Interferometry of p53 binding to immobilized TOE1

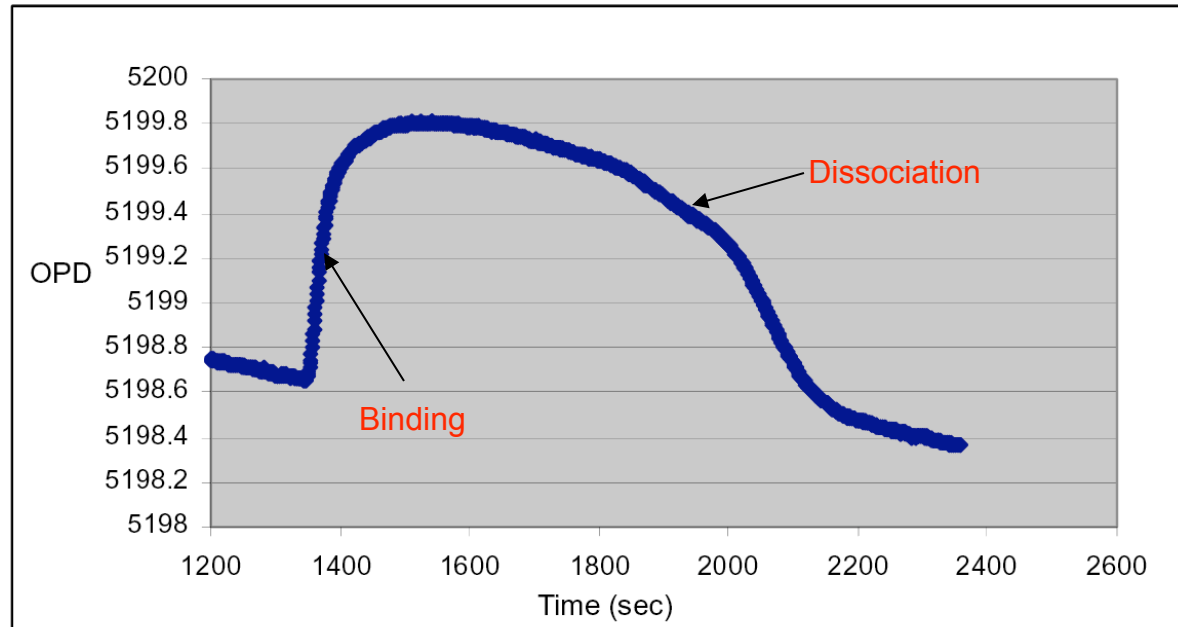


Figure 5

TOE1 was immobilized to a nanoporous silicon chip at 0.02 mg/ml using sulfo-NHS/EDAC chemistry. Binding was performed using full length wild type p53 at 0.01 mg/ml in a 100 μ l injection loop at a flow rate of 10 μ l/min using the Silicon Kinetics SKiProTM platform equipped with the flow cell format.

Nanoporous Interferometry of C terminal p53 binding to immobilized TOE1

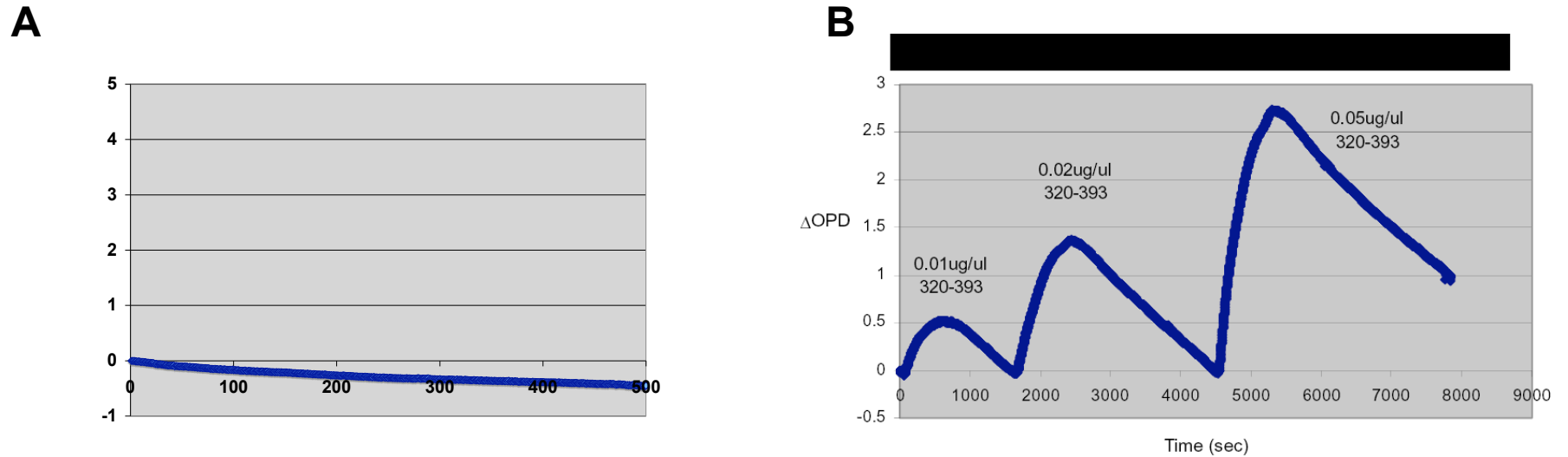


Figure 6

TOE1 was immobilized as in figure 4. In panel A, GST-p53 containing the N terminal 1-160 residues was provided at 0.01 mg/ml as in figure 4. In panel B, successive injections of the indicated amount of GST-p53 containing the C terminal 320-393 residues was provided for binding. Experiments were performed on a Silicon Kinetics SKiPro™ instrument equipped with the flow cell format.

These data were used to calculate the dissociation constant $K_D = 734$ nM.

Transactivation of the p21 and PTEN promoters by p53 and TOE1

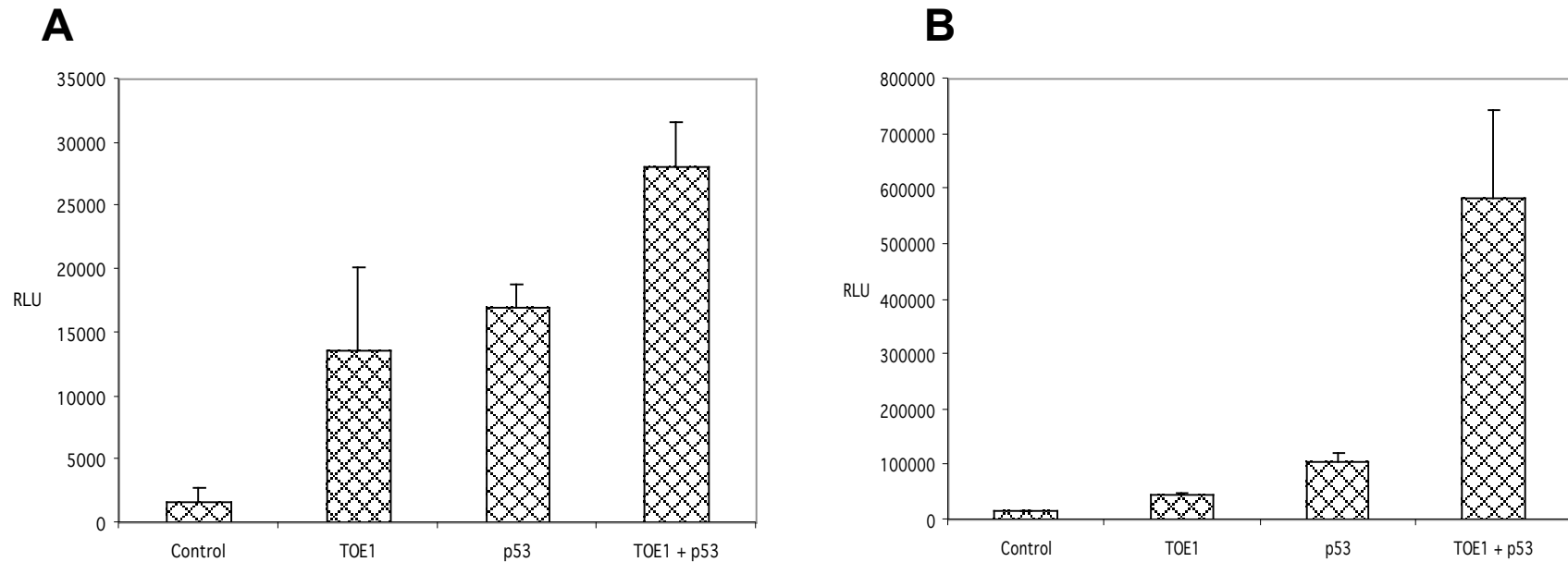
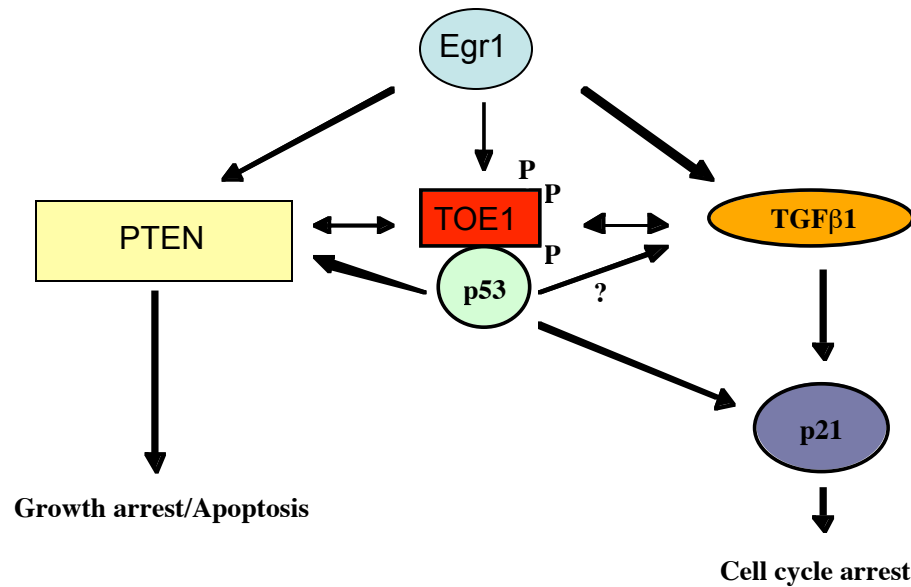


Figure 7

293 cells were transfected with a luciferase reporter containing the p21 (A), or PTEN (B), promoter together with the indicated expression vectors. Control samples were transfected with empty expression vector. 24 hours after transfection, cells were harvested and assayed for luciferase activity by luminometry. Results are presented as the average of triplicate samples with standard deviations indicated by error bars.

* p53 and TOE1 can activate transcription cooperatively

Model for TOE1 as a co-activator of gene expression leading to growth suppression



Cooperation between Egr1 and p53 in activating target genes leading to growth/cell cycle arrest. Egr1 and p53 both activate PTEN, TGFβ1 and p21 genes. TOE1 is shown as an Egr1 target gene that is a phosphoprotein binding to and increasing the activity of p53. In this way, Egr1 enhances the cooperative effect with p53 by inducing its activator.