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Expression of p300-truncated fragments results in the modulation of apoptosis in rat mesangial cells

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Background. Mesangial cell proliferation, apoptosis, and matrix deposition have pivotal roles in the pathogenesis of renal diseases such as diabetic nephropathy and glomerulonephritis. The behavior of mesangial cells depends on the integration of intracellular signals elicited by hormones and cytokines. We hypothesized that p300 is primarily involved in the integration of signal transduction pathways in rat mesangial cells (RMCs) and that interference with p300 function will alter apoptotic signals.

Methods. We established an RMC cell line expressing the Tet-activator (tTA). RMC-tTA cells were transiently transfected with vectors coding for either the N-terminal third or the C-terminal third of p300. Expression was induced by the addition of doxycycline [Dox; 1 μg/mL; 5% fetal bovine serum (FBS)]. The percentage of apoptosis was determined using the TUNEL technique. Specific protein–protein interactions were determined by Western blot analysis of immunoprecipitated complexes. Cells were treated with 5% FBS or with H_2O_2 (500 μmol/L, 1 h) with and without Dox.

Results. The expression of p300-C resulted in increased susceptibility to low serum-induced (20.0 ± 4.6 vs. 3.0 ± 1.7%) and to H_2O_2-induced apoptosis (75.3 ± 13.3 vs. 50.8 ± 6.5%) compared with controls. Immunoprecipitation of p300-C showed an interaction with the transcription factor c-Fos, which was enhanced by H_2O_2 treatment. Expression of the p300-N resulted in a rescue (34.8 ± 6.4 vs. 50.8 ± 6.5%) from H_2O_2-induced apoptosis compared with controls. P300-N was shown to form a complex with the transcription factor nuclear factor-κB (NF-κB).

Conclusions. The data indicate that endogenous p300 is involved in apoptosis in mesangial cells. We propose that interference or enhancement of endogenous p300 function, by expression of exogenous fragments, can alter interactions with c-Fos or NF-κB and modulate signals during cellular stress.

Glomerular mesangial cells play a central role in the pathogenesis of many important forms of kidney disease, such as chronic glomerulonephritis, diabetic nephropathy, and nephrosclerosis. Examples of altered mesangial cell behavior include increased matrix deposition in diabetic nephropathy [1], increased proliferation in chronic glomerulonephritis, and decreased apoptosis in lupus nephritis [2]. The altered behavior is not a consequence of a primary disease process in the mesangial cells, but rather a response to extracellular signals mediated by hormones, cytokines, and matrix components [3]. Transcriptional stimulation in response to cytokines, stress, retinoids, and steroids is mediated through the p300 protein [4–6]. p300 and its closely related cAMP-responsive element binding protein (CREB)–binding protein, CBP, are ubiquitous, evolutionarily conserved proteins that function, at least in part, by linking several different signal responsive transcriptional activators to the basal transcription machinery [7–11]. In addition, p300/CBP appears to serve as a switching molecule involved in integrating multiple signal transduction pathways within the nucleus. Activation of cellular Ras with insulin or nerve growth factor (NGF) results in the recruitment of the S6 kinase $pp90^{rsk}$ into a complex with p300/CBP [12]. In turn, the $pp90^{rsk}$-p300/CBP complex represses transcription of cAMP-responsive genes. Inasmuch as the data indicate that p300/CBP determines which transcriptional pathways will be activated, the fidelity of its function is vital for the maintenance of tissue integrity.

We propose that under pathologic conditions, when an abundance of mediators simultaneously engage a multitude of intracellular pathways, signaling fidelity would be compromised and that the cell would receive mixed signals, that is, activation of signals through the cAMP pathway coincident with activation of signals through the Ras pathway. This could result in loss of homeostasis, manifested as aberrant transcriptional changes, proliferation, and/or death.

Based on this idea, we hypothesized that p300 mediates cellular signals to undergo apoptosis in mesangial cells and that modulation of p300 function will block cell death

Key words: c-Fos, NF-κB, p300 protein, apoptosis, mesangial cells.

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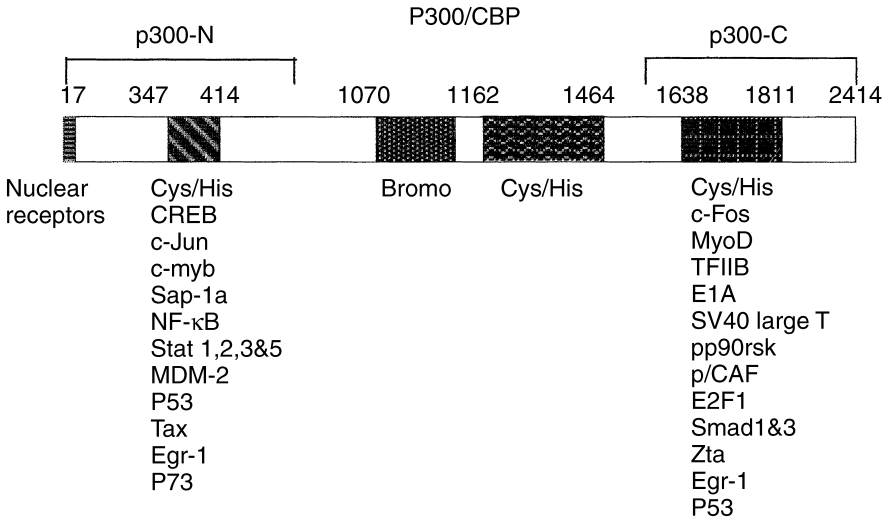

Fig. 1. A schematic representation of p300/CBP and reported proteins that interact with either the N-terminal or C-terminal portions of the molecule.

signals. To test this, disruption/modulation of p300 function was achieved by exogenously expressing p300 fragments, with the assumption that the fragments would compete with endogenous p300 for binding to endogenous transcription factors. We have employed a tetracycline-inducible system for the expression of the p300 fragments [13]. In vitro, the tetracycline-responsive system has been shown to be advantageous for gene expression studies [14], and in vivo, it has been used as an approach for gene targeting in the renal glomerulus [15]. Once the gene of interest is subcloned into the appropriate vector, activation is achieved by the addition of tetracycline or doxycycline (Dox), to a previously established clonal cell line expressing a bacterial tetracycline repressor fused to a viral VP16 transactivator, tTA. In the presence of Dox, the Tet-On activator will bind to sequences containing a Tet-operator (Tet O) juxtaposed to a minimal promoter.

In the studies presented here, hydrogen peroxide (H_2O_2) was used as a signal inducer, chosen on the basis of published data showing that H_2O_2 plays a significant role in glomerular disease [16–18]. Stimulation of a H_2O_2-responsive signal transduction pathways can result in activation of transcription factors, including nuclear factor-κB (NF-κB) and c-Fos, in glomerular mesangial cells [18, 19].

Nuclear factor-κB, which binds the N-terminal domain of p300 (Fig. 1) [20] is a heterodimeric protein composed of 50 and 65 kD subunits. The p65 subunit (RelA) interacts with p300 [6]. NF-κB is an inducible transcriptional activator critical in the regulation of gene expression in response to injury and inflammatory stimuli [21].

c-Fos, which binds the C-terminal domain of p300 (Fig. 1) [22], is a member of the activator protein-1 (AP-1) transcription factor complex. In addition to a primary role in normal development and cellular growth, c-Fos protein has been associated with apoptotic cell death induced in response to cellular injury [23, 24]. In our earlier studies, we showed that c-Fos is actively involved in the activation of apoptosis in a p53-dependent pathway [25]. A role for p53 in mesangial cell apoptosis has been implicated in response to superoxide radicals, and the p53 pathway has been linked to p300/CBP function [26, 27].

We report the novel finding that overexpression of the C-terminal fragment of p300 in RMC-tTA cells resulted in an increased sensitivity to low serum- and H_2O_2-induced apoptosis. Our results suggest that stress-induced changes in mesangial cells in a diseased environment can be modulated by interference with transcription factor–p300 interactions.

METHODS

Establishment of Tc-responsive p300 vectors

The spontaneously immortalized rat mesangial cell line RMC was a generous gift from Dr. R.J. Johnson (University of Washington Medical Center, Seattle, WA, USA). The complete cDNA of p300, provided in a CMVβ vector, was a gift from Dr. Richard Eckner (Dana-Farber Cancer Institute, Seattle, WA, USA). A 3134 bp fragment, comprising nucleotides 5913 to 9046 (aa 1572 to 2414) was generated by endonucleases Sma I and HIND III (New England Biolabs, Beverly, MA, USA). A fragment of 2229 bp, comprising nucleotides 1200 to 3429 (aa 1 to 743), was generated by endonucleases Sac I and Sma I (New England Biolabs). Purified fragments were blunted and cloned into the EcoRI site of the pTRE expression vector (Clontech, Palo Alto, CA, USA). A correct sequence of constructs, pTRE-Dp300C and pTRE-Dp300N, was confirmed by sequencing.

Cell culture

The RMC-tTA cell line was cultured in RPMI 1640 medium (GIBCO BRL, Grand Island, NY, USA), supplemented with bovine insulin (0.67 U/mL; GIBCO BRL), sodium pyruvate (89 μg/mL; GIBCO), L-glutamine (200 mmol/L; GIBCO), sodium bicarbonate solution 7.5%, HEPES (10 mmol/L), fetal bovine serum (FBS; 18%; Sigma), and 400 μg/mL G418 (GIBCO) and was incubated at 37°C in 10% CO_2 in air.

Generation of tTA-expressing (Tet-On) clones

Rat mesangial cells were trypsinized and replated 24 hours prior to transfection. The Tet-On vector was transfected into RMCs by electroporation, which was performed in serum-free medium with 300 V at a capacitance of 960 μF (Gene Pulser, BRL, Grand Island, NY, USA). Transfection efficiencies determined by staining cells transfected with the β-galactosidase gene ranged from 25 to 40%. For each transfection, 8×10^6 cells were mixed with 30 μg of DNA in a volume of 0.6 mL. G418-resistant clones were expanded, and the integrity of the regulating element was tested using a luciferase gene reporter assay, the Promega Luciferase Assay System (Promega Corp., Madison, WI, USA). Out of 40 tested clones, 8 exhibited differential expression of luciferase proportional to the Dox concentration in the medium. The selected clones were maintained in medium containing G418 to preserve the indelibility. Long-term stability was not determined since cell cultures had not been maintained for more than three months. In addition, Western analysis of protein expression was performed using Dox at concentrations of 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, and 7.5 μg/mL. Cells were transfected with pTRE-Dp300C and incubated for 24 hours in medium plus 18% FBS. The medium was changed to 5% FBS plus or minus Dox, and the cells were incubated an additional 16 hours. Cell lysates were prepared for protein analysis.

Protein extraction and immunoblotting

Cells were incubated without or with Dox (3 μg/mL in 18% FBS or 1 μg/mL in 5% FBS) for 16 to 18 hours. Adjusted Dox concentrations reflected the reduced total protein in the culture medium. Cells were rinsed twice in cold PBS. A volume of sodium dodecyl sulfate (SDS)-polyacrylamide gel Laemmli sample buffer (100°C) was added directly to cells to achieve a final concentration of 3×10^6 cells/mL. Cell lysates were collected, boiled for five minutes, and sonicated. Aliquots equal to 2×10^5 cells were subjected to SDS-polyacrylamide gel electrophoresis at 150 V for one hour and transferred to nitrocellulose at 100 V for 45 minutes in transfer buffer.

Analysis of immune complexes by immunoprecipitation

Rat mesangial-tTA cells were transiently transfected, and after 24 hours were placed in Dox for 18 hours. Cells were lyzed in modified NP-40 lysis buffer [25 mmol/L HEPES (pH 7.5), 12.5 mmol/L $MgCl_2$, 150 mmol/L KCl, 0.5% NP-40, 1 mmol/L dithiothreitol] containing 10 μg of leupeptin per mL, 1% aprotinin, and 0.5 mmol/L phenylmethylsulfonyl fluoride. Glycerol was added to a final concentration of 10%. After incubation (1 h at 4°C), extracts were centrifuged at 6000 r.p.m. for 15 minutes, and supernatants were collected. The precleared extract was incubated with the anti–c-Fos antibody or anti–NF-κB antibody overnight at 4°C. Immune complexes were precipitated using protein A-agarose. Pellets were washed three to five times in lysis buffer and eluted in 1.5 × Lammeli sample buffer. Samples were analyzed by Western blot.

Antibodies

Anti-p300 mouse monoclonal antibody (Power-clone), specific for the C-terminal portion of endogenous p300, was purchased from Upstate Biotechnology (Lake Placid, NY, USA). Rabbit polyclonal anti–N-terminal p300, polyclonal anti–C-terminal p300, polyclonal anti–c-Fos, and polyclonal NF-κB antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Analysis and quantitation of apoptosis

Rat mesangial-tTA cells were transfected with vectors pTRE-Dp300C, pTRE-Dp300N, and pTRE-Dp-luciferase. DNA fragmentation was analyzed on combined attached and detached cells using an apoptosis detection kit from Pharmingen (San Diego, CA, USA). In brief, apoptotic cells were labeled by the catalytic incorporation of fluorescein-12-dUTP at the 3′-OH ends using the enzyme terminal deoxynucleotidyl transferase. Positive cells were quantitated by flow cytometry. Clumps and doublets were excluded from the analysis by using forward scatter versus side scatter.

RESULTS

Modifiable expression of the p300-N and -C terminal fragments in rat mesangial-tTA cells

To determine the efficacy of the Dox-inducible system in mesangial cells, the first step was to establish a clonal cell line of RMCs that expressed the tetracycline (or Dox) transactivator (tTA) protein. The tTA vector was transfected into RMCs. Forty G418 resistant clones were isolated. Clones were transfected with a luciferase reporter construct and evaluated for controlled expression of luciferase proportional to Dox concentration and eight clones were identified. Figure 2A shows that protein expression levels are proportional to the Dox concentration (lanes 1 through 8 at doses of 0 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 0.75 μg/mL, 1.0 μg/mL, 2.5 μg/mL, 5.0 μg/mL, and 7.5 μg/mL) in clone A14, the clone used in the studies presented here. Substantial induction was

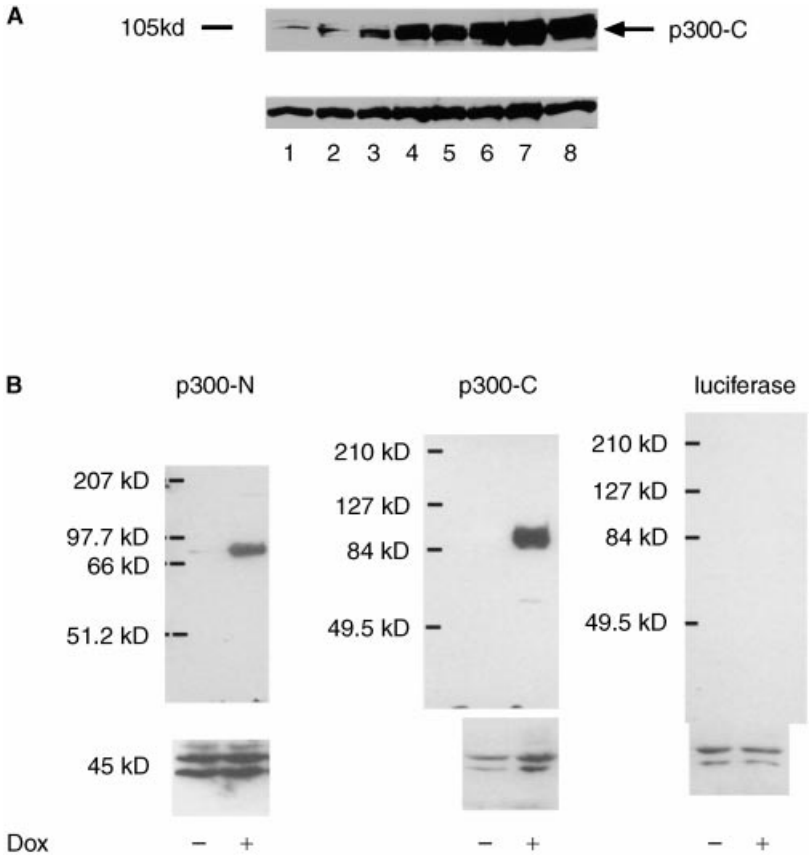

Fig. 2. (*A*) Protein expression levels of p300-C are proportional to the doxycycline (Dox) concentration. Lane 1, 0 μg/mL; lane 2, 0.25 μg/mL; lane 3, 0.5 μg/mL; lane 4, 0.75 μg/mL; lane 5, 1.0 μg/mL; lane 6, 2.5 μg/mL; lane 7, 5.0 μg/mL; and lane 8, 7.5 μg/mL. Protein loading controls are shown below. (*B*) Western blot analysis showing inducible expression of p300-N (probed with N-terminal specific antibody), p300-C (probed with p300-C specific antibody), and luciferase-expressing controls (probed with p300-N specific antibody) in RMC-tTA cells, with and without Dox. Western blots were standardized by cell number (1.2×10^5 per lane). Blots were reprobed for ERK kinase (p44 to p46) to determine loading efficiency.

observed at concentrations higher than 0.75 μg/mL (lane 4) up to 5 μg/mL (lane 7), but decreased at concentrations that approached toxic levels of 7.5 μg/mL (lane 8). No obvious induction was detected in RMC-tTA in the absence of Dox. The slight band that is observed in lane 1 is a nonspecific band seen also in nontransfected cells (data not shown). Included below the p300-C Western blot is a control band to show protein loading. Thus, modest concentrations of Dox were sufficient to activate the exogenous gene activity of the pTRE-Dp300C vector.

The immunoblots in Figure 2B show that Dox treatment (1 μg/mL) of transiently transfected RMC-tTA cells induced expression of the p300-N (80 kD; aa 1-743) by 20-fold compared with transfected cells incubated without Dox. Induction of the p300-C (97 kD; aa 1572-2414) resulted in a 25-fold increase in protein levels (Fig. 2B). Cells transfected with the pTRE vector containing the luciferase gene were analyzed as controls (Fig. 2B). The blots were reprobed with an anti-ERK (45 kD) antibody to show protein loading.

Functional role of p300-N and p300-C in low-serum–induced apoptosis

During the course of establishing the effective concentrations of Dox, the cells were placed in 5% serum in order to keep the Dox concentration at a minimum (Dox effectiveness is proportional to total protein concentration). We observed that the original RMC population and the RMC-tTA cells were sensitive to decreased concentrations of FBS. At 5% FBS, there was considerably more death in the culture. Also, it appeared that cell cultures induced to express p300-C showed many more detached cells, as compared with transfected cells without Dox. To test this specifically, transfected RMC-tTA cells were placed in 18 or 5% serum with and without Dox (3 and 1 μg/mL, respectively). Dox-induced expression of the p300 fragments had no effect on cell viability when the cells were incubated under normal proliferative conditions of 18% FBS. In two separate experiments, <1% TUNEL-positive apoptotic cells was detected in

Table 1. Percent apoptosis in transiently transfected RMC-tTA cells treated with DOX (1 μmol/L) for 18 hours

Treatment	Luciferase +DOX	P300-N +DOX	P300-C +DOX
Low serum	3.0 ± 1.7%	5.8 ± 3.7%	20.0 ± 4.6%
H_2O_2	50.8 ± 6.5%	34.8 ± 6.4%	75.3 ± 13.3%

Values were normalized to Luciferase vector controls minus doxycycline (DOX) of 5% death in low serum and 50% death with H_2O_2-treatment. Values were not adjusted for transfection efficiencies, ranging from 25 to 40%. Each value is the mean ± standard deviation of three independent experiments.

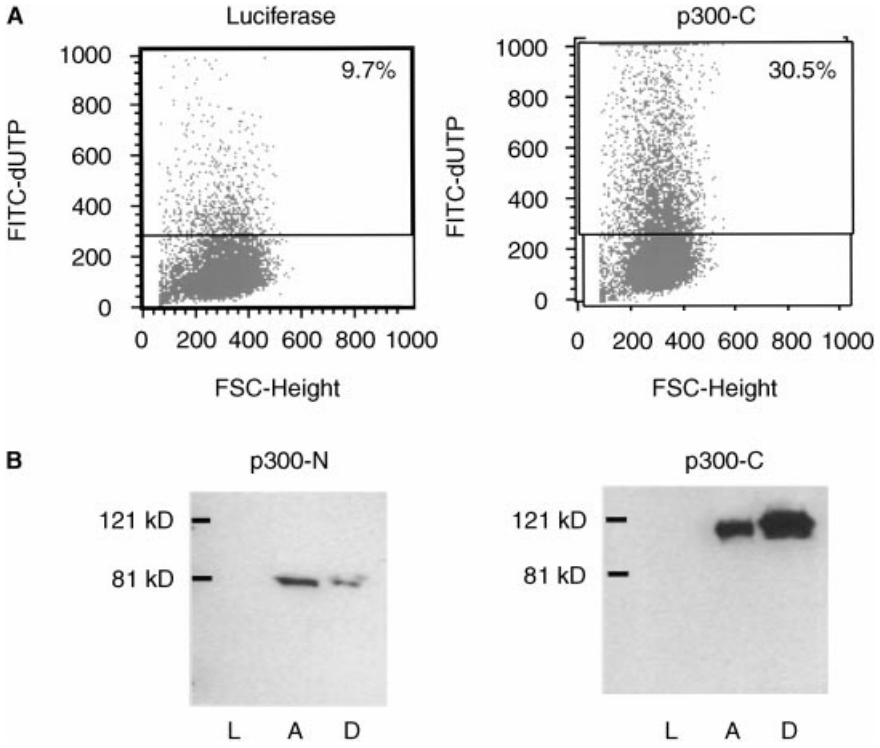

Fig. 3. P300-C expression results in increased apoptosis under low serum conditions. (*A*) A typical flow cytometry analysis (FACScan) of apoptotic cells, plotted as FITC-dUTP (TUNEL positive), verses forward scatter. Cells were treated with Dox 18 hours in 5% FBS. (*B*) Western analysis: Lane 1 contains cell lysates from luciferase vector transfected controls. Lane 2 contains attached cell lysates. Lane 3 contains lysates from cells detached at the time of harvest.

all groups (data not shown). Evaluation of cells placed in 5% FBS showed that expression of the p300-N fragment had no significant effect on cell death (Table 1), compared with luciferase transfected controls (3.0 ± 1.4% vs. 5.8 ± 3.7%, respectively). In contrast, expression of p300-C resulted in an increase in apoptosis to 20.0 ± 4.0%. A typical flow cytometric analysis of apoptotic cells is shown in Figure 3A as a dot blot, plotted as TUNEL positive (FITC-dUTP) cells versus forward scatter (FSC-H). When considering that the transfection efficiencies ranged from 25 to 40%, an increase of approximately 15% is substantial. In addition, Western analysis (Fig. 3B) showed that a large fraction of cells expressing the p300-C protein were found in the detached (D) population, whereas p300-N protein was found primarily in the attached (A) population. Therefore, the data are consistent with the proposal that p300-C, but not p300-N, is involved in low serum-induced apoptosis in RMCs.

p300-N and p300-C participate in H_2O_2-induced apoptotic pathways

To establish the sensitivity of RMC-tTA cells to H_2O_2, the cells were treated with increasing concentrations of H_2O_2, and apoptosis was measured by TUNEL analysis. As shown in Figure 4, at 500 μmol/L of H_2O_2, approximately 40 to 60% of the cells underwent apoptosis. At a concentration of 700 μmol/L, there were fewer TUNEL-labeled cells. We attribute this to increased necrosis at this dose, based on the morphologic appearance of the

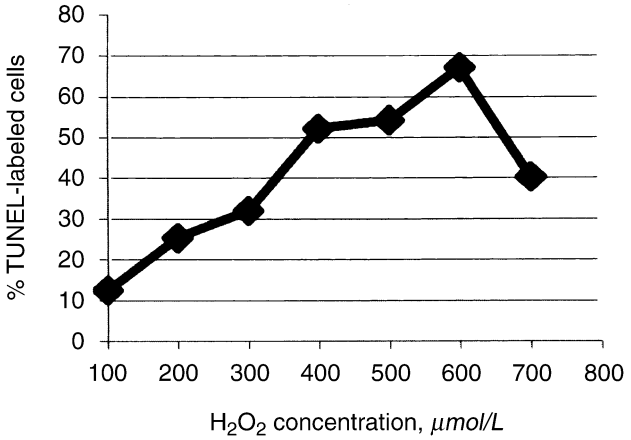

Fig. 4. Percent TUNEL-labeled apoptotic RMC-tTA cells incubated in varying concentrations of H_2O_2 for one hour.

dying cells. RMC-tTA cells were transfected, incubated with Dox plus 5% FBS, and then treated with H_2O_2 (500 μmol/L 1 h). Forced expression of the p300-N fragment resulted in a 30% reduction in H_2O_2-induced apoptosis compared with luciferase transfected controls (Table 1). In contrast, induced expression of p300-C resulted in an approximately 50% increase in H_2O_2-induced apoptosis from 50 to 75% as compared with Dox treated, luciferase-expressing controls. Figure 5A shows a typical flow cytometric analysis of p300-C transfected cells exposed to H_2O_2. Minus Dox shows 34% TUNEL-positive cells,

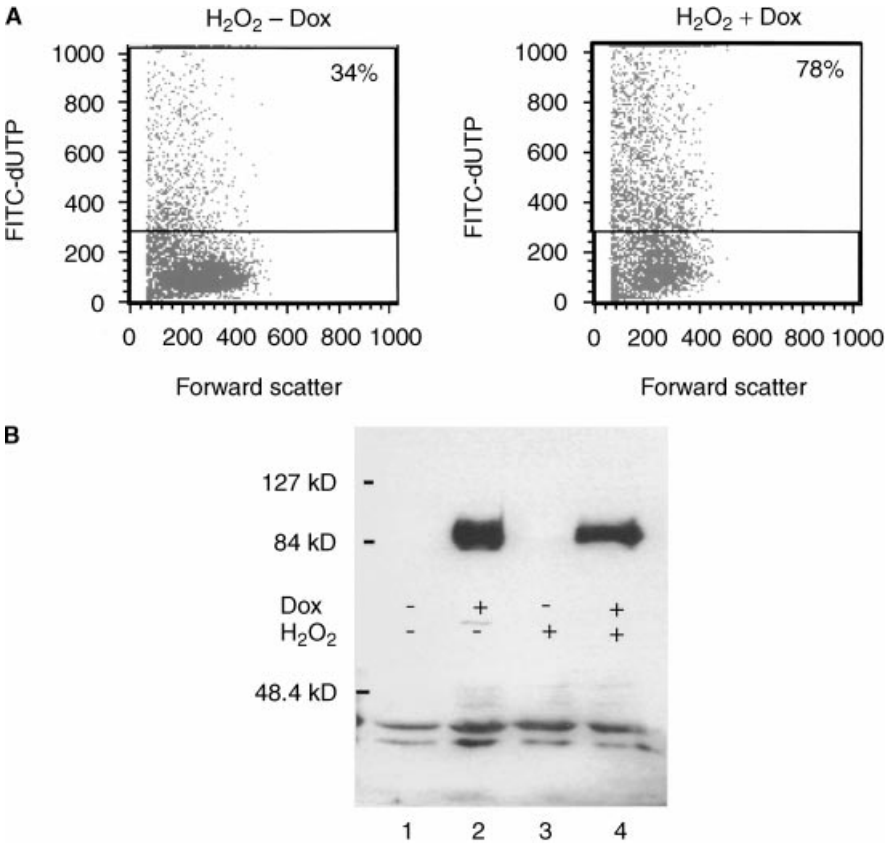

Fig. 5. P300-C expression results in increased apoptosis in the presence of H_2O_2 (500 μmol/L for 1 h). (*A*) Typical FACScan analysis of apoptotic cells, plotted as FITC-dUTP (TUNEL positive) versus forward scatter, comparing percentage death minus Dox versus plus Dox. (*B*) Western analysis of p300-C with and without H_2O_2. Blot was reprobed for ERK kinase (pp4 to p46) to show protein loading.

whereas the addition of Dox resulted in 78% positive cells. To assure that H_2O_2 treatment did not interfere with the regulation of the pTRE vectors, Western blot analysis for protein expression was performed. H_2O_2 treatment did not alter the Dox-induced expression of p300-C (Fig. 5B) or p300-N (data not shown).

Association of p300 fragments with endogenous transcription factors

It was important to know whether the exogenously expressed p300 fragments were binding to the endogenous transcription factors. To demonstrate the physical association of p300-C with c-Fos, we used immunoprecipitation-Western blot analysis to detect interactions. Extracts from cells, transiently transfected with a p300-C expression vector (minus Dox/plus H_2O_2 and plus Dox plus H_2O_2), were immunoprecipitated using a p300-C terminal-specific antibody. The transcription factor c-Fos was detected in immune complexes precipitated with a C-terminal–specific p300 antibody, but only in the presence of Dox (Fig. 6A). These data indicate that endogenous c-Fos physically interacts with the recombinant C-terminal portion of p300. The same approach was taken to detect NF-κB/p300-N interactions using a p300 N-terminal–specific antibody. Western blot analysis of immunoprecipitated complexes revealed that NF-κB was present in the p300-N complex (Fig. 6B). Therefore, human/p300 fragments can associate with rat endogenous transcription factors in intact cells.

DISCUSSION

This study introduces a new concept in the study of intracellular signaling in mesangial cells. The p300 protein is well known to bind multiple transcription factors in the C-terminal and N-terminal domains of the molecule (Fig. 1). We report the novel finding that p300 is involved in H_2O_2 signaling pathways in mesangial cells and that these signals can be modulated by forced expression of p300 truncated fragments. Both the N-terminal and the C-terminal fragments of p300 were shown to interact with endogenous transcription factors, and both fragments were functionally active. Expression of the N-terminal fragment rescued cells from H_2O_2-induced apoptosis, while expression of the C-terminal fragment enhanced apoptosis.

The efficacy to the Tet-On system in RMCs has been demonstrated in these studies. The RMC-tTA cells produced a protein of the anticipated size in response to Dox that showed immunoreactivity with p300 antibodies. The recombinant proteins formed complexes with activated endogenous transcription factors. Apoptosis was

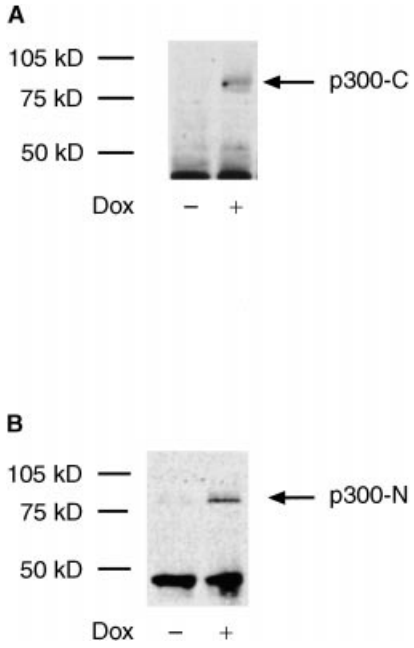

Fig. 6. p300-C binds c-Fos and p300-N binds NF-κB. RMC-tTA cells were treated with H_2O_2 for 15 to 30 minutes plus or minus Dox. (*A*) Western blot analysis of protein complexes immunoprecipitated with a c-Fos specific antibody from p300-C expressing cells. Western blot was probed with a p300-C-specific antibody. (*B*) Western blot analysis of protein complexes immunoprecipitated with a NF-κB specific antibody, from p300-N expressing cells. Western blot was probed with a p300-N-specific antibody.

modulated by the expression of p300 fragments. It will be interesting to determine whether overexpression of the whole p300 molecule will result in increased apoptosis in the RMCs once the gene is subcloned into the pTRE vector. We have shown that overexpression of p300 in p53 wild-type cells, including Syrian hamster embryo fibroblast cells and human tumor cells, induces apoptosis (Preston et al, manuscript submitted for publication). The use of the Tet-On system will enable us to introduce varying amounts of the p300 molecule and the p300 fragments into mesangial cells that could interact with transcription factors, thus modulating the response to signaling pathways.

It was difficult to predict whether the complexation of the p300 fragments with transcription factors would lead to attenuation of a response due to sequestration or whether a response would be augmented due to increased amounts of a complex with biological activity. It has been reported that preventing AP-1 activation can block mesangial cell apoptosis [28]. Unpublished data, which indicated that AP-1 was involved in our system, showed that c-Fos (a member of the AP-1 transcription factor complex) levels were increased in the presence of H_2O_2, as determined by TaqMan quantitative reverse transcription-polymerase chain reaction. As such, we anticipated that titration of c-Fos would result in decreased apoptosis; however, the opposite effect was observed. There are at least three possible explanations for the finding of enhanced death in the presence of p300-C: c-Fos complexes. First, the truncated fragment may have unimpaired the ability to initiate transcription, assuming that the loss of two thirds of the molecule may simply mean that the regulatory portions of the molecule are no longer present. However, it is intriguing that the N-terminal fragment seemed to also be able to form functional complexes. A second possibility is that the c-Fos/p300-mediated apoptosis is a nontranscriptional event, but possibly an activation event leading to eventual triggering of caspases. This concept is supported by our earlier work showing that c-Fos–induced apoptosis was a nontranscriptional event [25]. Third, the levels of endogenous p300 are strictly controlled, but it is not known how they are kept constant. It is reasonable to assume that overexpression of recombinant fragments may alter the homeostasis of the endogenous proteins. In fact, in many experiments the transfection of the N-terminal fragment appeared to decrease the levels of endogenous p300 (data not shown) even though this was not studied systematically. It is possible that generation of endogenous p300 fragments is required as part of the normal apoptotic process. In support of the existence of endogenous p300 fragments, a published report has described a subspecies (fragment) of p300 (a C-terminal portion) that could be immunoprecipitated with the polyomavirus large T antigen [29]. Our data would imply that this subspecies might have biological activity.

Two published reports have described phenotypic changes associated with exogenously expressed p300-C-terminal fragments. Fujii et al microinjected RNA of the carboxy terminal-truncated portion of *Xenopus* p300 (Xp300) into a blastomere stage embryo and found that it invoked the malformation of the embryo [30]. Avantaggiati et al showed that cotransfection of p53 in combination with the C-terminal fragment of p300 blocked p53-induced apoptosis [31]. These data are in contrast to our findings of increased death with expression of the p300-C-terminal fragment. One explanation would be that their experiments involved overexpression of p53, while our experimental system dealt with endogenous levels of p53 with overexpression of portions of p300. We have not studied p53 in our system as it relates to overexpression of p300 fragments; however, overexpression of p300 enhances E1 A-induced apoptosis [32]. Also, Avantaggiati et al used the SAOS human tumor cell line that is $p53^{-/-}$, as well as $Rb^{-/-}$. The precise cohort of genes altered in these tumor cells and the signaling pathways that have been inactivated/activated will be different, and it is unlikely that this cellular system would respond similarly to our system.

The importance of p300 in the regulation of cellular homeostasis is reflected in the fact that multiple viral

proteins have been shown to bind and to modulate p300 function. As shown in Figure 1, these include adenoviral protein E1 A [7], SV40 large T protein [33], Ebstein Barr viral protein, zta [34], and T-cell leukemia virus type 1 Tax protein [35]. p300/CBP also seems to be involved in the mechanism that senses and controls the repair of DNA damage. Expression of proliferating cell nuclear antigen (PCNA), a crucial component for DNA polymerase activity during repair, is activated through a p300/CBP-dependent mechanism [36].

Our results with H_2O_2 provide a conceptual basis for utilization of this system to determine if other signaling pathways, such as tumor necrosis factor-α, interferon-γ, or transforming growth factor-β, can be modulated by expression of p300 fragments. These cytokines are known to activate transcription factors during disease states. The Smad family of transcription factors, which interact with p300/CBP (Fig. 1), are intermediate effector proteins that transduce transforming growth factor-β signals [37]. Interferon-γ stimulates the activity of the Stat family of transcription factors, which also bind p300 (Fig. 1) [38].

In summary, the new findings outlined here indicate that p300 is an important signaling molecule in mesangial cells. Utilization of the RMC-Tet-On system will be informative for studies of disease states that alter the ability of the transcription factors to interact with p300 and to determine what effect this might have on the activation of gene expression.

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