Inhibition of protein tyrosine phosphatases causes phosphorylation of tyrosine-331 in the p60 TNF receptor and inactivates the receptor-associated kinase

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Abstract Inhibition of protein tyrosine phosphatases blocks tumor necrosis factor (TNF)-induced growth modulation and NF-κB activation, both mediated primarily through the p60 TNF receptor. How inhibition of the phosphatases affects the p60 TNF receptor or the recently described receptor-associated serine/threonine kinase (p60TRAK) is not known. In this report, we show that this inhibition, when induced by pervanadate, caused the tyrosine phosphorylation of the cytoplasmic domain (CD) of the p60 receptor, as revealed by phosphoamino acid analysis. Furthermore, site-directed mutagenesis indicated that pervanadate specifically induced the phosphorylation of tyrosine-331, which is located in the death domain of the TNF receptor, a domain to which p60TRAK binds. This tyrosine residue was also phosphorylated by purified, recombinant pp60^{Src} in vitro. Inhibition of protein tyrosine phosphatases by pervanadate also led to the inactivation of p60TRAK. In contrast, okadaic acid, a specific inhibitor of protein serine/threonine phosphatase, increased p60TRAK activity. Taken together, these results suggest that protein tyrosine phosphatases play an essential role in phosphorylation of the cytoplasmic domain of the TNF receptor and in regulation of the receptor-associated kinase, and this in turn may play a role in TNF-mediated growth modulation and NF-κB activation.

Key words: TNF receptor; Phosphorylation; Kinase; p60TRAK; Phosphatase

1. Introduction

The intracellular domains of both the p60 and p80 tumor necrosis factor (TNF) receptors lack motifs characteristic of protein tyrosine and serine/threonine kinases. Even so, several cellular proteins are phosphorylated both at serine/threonine and tyrosine residues upon engagement of the TNF receptor, including heat shock protein 27 [1], epidermal growth factor receptor [2], eukaryotic initiation factor 4E [3], IκBα (the inhibitory subunit of NF-κB) [4], insulin receptor substrate-1 [5–7], and the mitogen-activated protein kinase family (for references see review [8]). The increased protein phosphorylation could be due to the reported activation of a variety of kinases and/or the inhibition of protein phosphatases (PPase) (for references see review [9]).

By using the glutathione-*S*-transferase (GST) fusion protein system, we have previously demonstrated that two different serine/threonine protein kinases, p60TRAK and p80TRAK, associate with the cytoplasmic domains of the p60 and p80 TNF receptors, respectively [10–12]. Furthermore, a region of ~54 residues (344–397) within the death domain of the p60 receptor was found to be sufficient to associate with p60TRAK [12]. Additionally, a sequence residing near the C-terminus (residues 397–426), designated the kinase inhibitory domain (KID), inhibits the binding of p60TRAK to the cytoplasmic domain [12].

While protein kinases are evident in the TNF signalling pathway, the role of PPases is less well understood. However, recent reports indicate that a protein tyrosine phosphatase (PTPase) is involved in TNF-mediated cellular responses including cell growth inhibition and proliferation [13] and NF-κB activation [14–16], all mediated through the p60 form of the TNF receptor [17,18]. Whether the p60 TNF receptor and the associated kinase (p60TRAK) are also regulated by tyrosine phosphorylation is not known. In the present study, we show that inhibition of PTPase causes a protein tyrosine kinase to bind and phosphorylate tyrosine-331 which is located in the death domain of the TNF receptor. Additionally, PTPase inhibition results in inactivation of p60TRAK activity, and this may play a role in TNF-mediated growth modulation and NF-κB activation.

2. Materials and methods

2.1. Materials

All reagents were obtained and prepared as previously described [10–12]. Purified, recombinant pp60^{Src} was provided by Dr. R. Budde (The University of Texas M.D. Anderson Cancer Center).

2.2. Construction of plasmids, expression and purification of fusion proteins and in vitro kinase assays

All plasmids encoding GST-p60 cytoplasmic domain deletions have been described previously [10,11]. The GST-p60CD fusions used in these studies included Δ8, Δ12, and Δ15, which encompass residues 324–397, 344–397, and 243–397, respectively [12]. The plasmids encoding site-directed mutants of GST-p60Δ8^{T329A}, GST-p60Δ8^{Y331F}, and GST-p60Δ8$^{T329A/Y331F}$ were generated by polymerase chain reaction using pCMVXVBpL4-p60 [10] as the template and the following primers: Δ8^{T329A} (5′-*Bam*HI): CTAAGAGGATCCACTGATGACC CCGCGGCGCTGTAC; Δ8^{Y331F} and Δ8$^{T329A/Y331F}$ (5′-*Bam*HI): CTAAGAGGATTCA CTGATGACCCCGCGG/ACGCTGTTCGC-CGTGG; and for all Δ8 mutants (3′-*Hin*dIII): TCTTAGAAGCTTT-TAGCGGAGCACGCGTCCCAG. The PCR products were digested with *Bam*HI-*Hin*dIII and inserted into pGEX2TH. The creation of a *Ksp*I site (underlined) in the primers made positive selection for the site-directed mutants possible.

Expression of all GST fusion proteins from BL21 cells harboring the appropriate expression vector was induced with 0.5 mM isopropylthiogalactoside at 15°C for 1 h and purified on glutathione-agarose beads as previously described [10,11]. Throughout this report, all fusion proteins will be referred to by their deletion number (i.e., GST-p60Δ8 will be referred to as p60Δ8). In vitro kinases assays were

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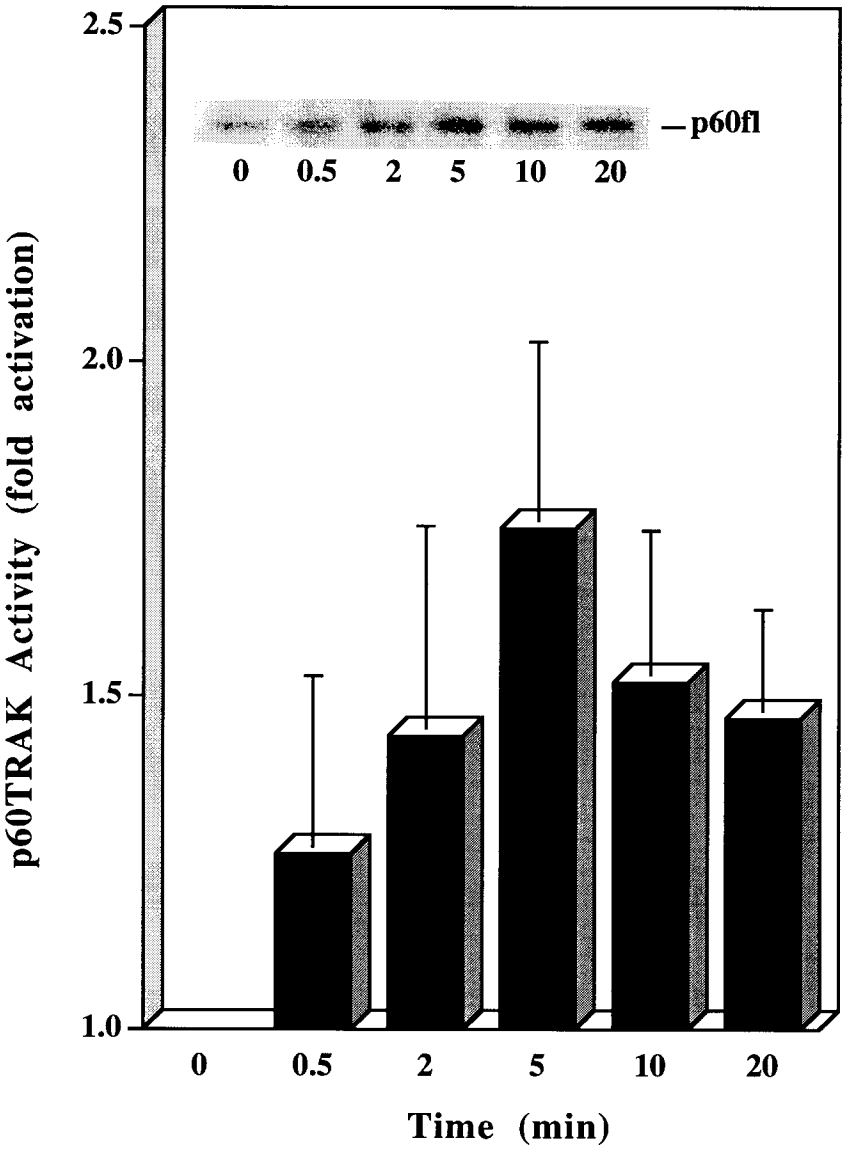

Fig. 1. TNF activates p60TRAK in a time-dependent manner. U937 cells were treated with 10 ng/ml TNF for the indicated times and lysates were prepared. Cell lysates (300 μg) were affinity precipitated with p60fl and in vitro kinase assays were performed. The phosphorylation of p60fl was quantitated by a Phosphorimager and ImageQuant software. The fold-activation is presented as the deviation from the mean of three independent experiments. One such experiment is shown in the inset.

performed as previously described [12]. Quantitation of phosphorylated proteins was performed by PhosphoImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

3. Results

Previously, we reported that inhibition of PTPases blocks TNF-induced growth modulation [13] and NF-κB activation [15,16], both mediated primarily through the p60 form of the TNF receptor. In this report, we examined the effect of inhibition of PTPases on the TNF receptor and the receptor-associated kinase.

3.1. p60TRAK is activated by TNF

We first examined the activation of p60TRAK by treatment of cells with TNF. The full length cytoplasmic domain of the p60 (p60fl) TNF receptor (residues 205–426) was fused to the C-terminus of GST and used to precipitate kinase activity form cytoplasmic extracts from U937 cells treated with 10 ng/ml TNF for various times. The phosphorylation of p60fl was quantitated to measure the activity of p60TRAK. The results shown in Fig. 1 indicate that TNF activated p60TRAK activity in a time-dependent manner. The activation could be seen as early as 30 s, peaked at 5 min and remained elevated at 20 min.

3.2. Inhibition of PTPases causes tyrosine phosphorylation of p60 TNF reporter

To explore the effect of PTPases, we used the PTPase inhibitor pervanadate, which has been shown to block TNF-induced NF-κB activation [14–16]. For these experiments, we also used constructs from the cytoplasmic region of the p60 receptor that comprised residues 324–397 (p60Δ8) and residues 344–397 (p60Δ12), both of which lack the KID and

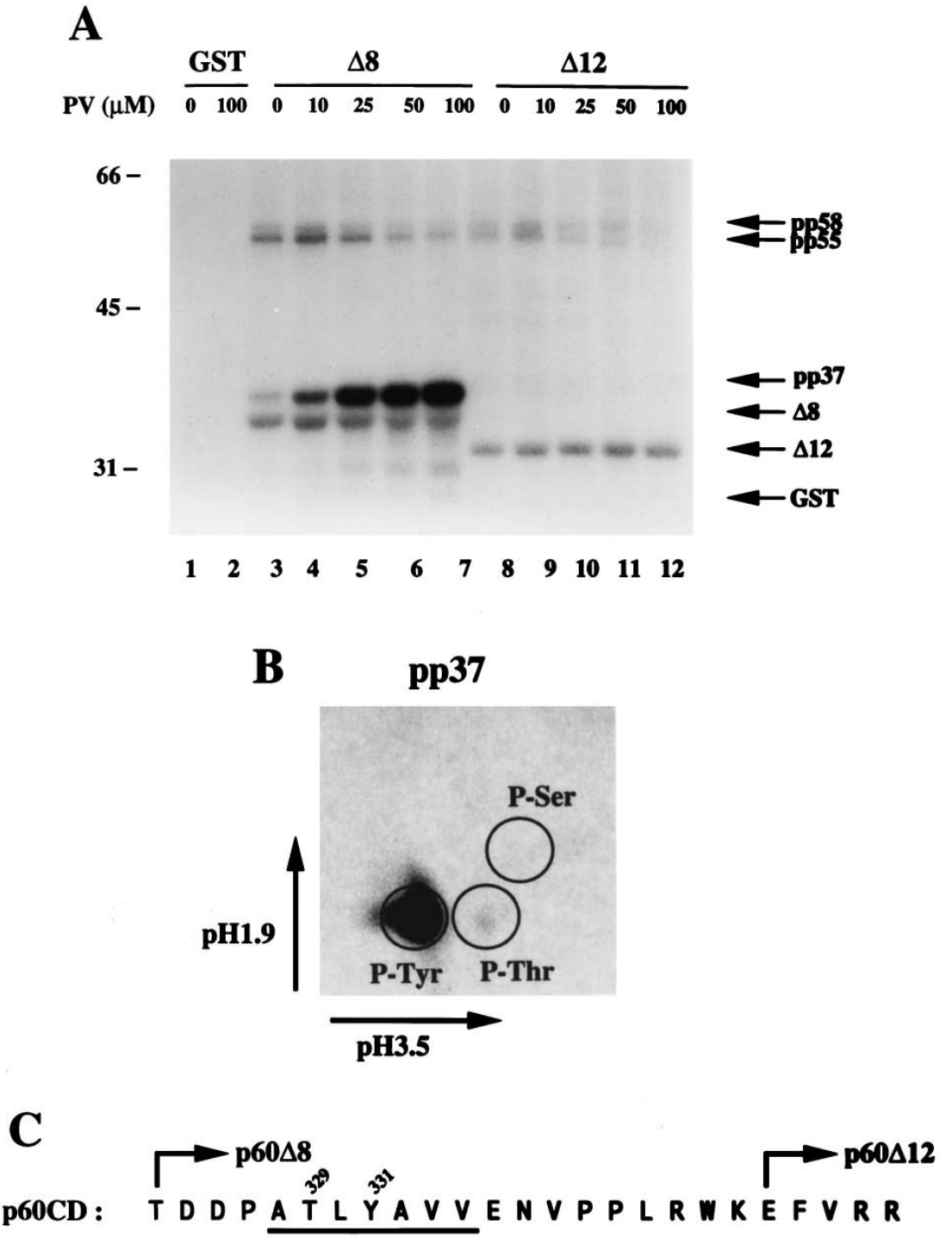

Fig. 2. Inhibition of PTPases by pervanadate causes tyrosine phosphorylation of the TNF receptor. (A) Effect of pervanadate on p60Δ8 and p60Δ12. U937 cells were incubated with the indicated concentration of the inhibitor for 30 min at 37°C. Cell lysates were prepared, and affinity precipitated, and in vitro kinase assays performed using GST, p60Δ8 and p60Δ12. The samples were analyzed by 9% SDS-PAGE, and the dried gel exposed to X-ray film for 8 h at −70°C. (B) Phosphorylated TNF receptor (pp37) contains only phosphotyrosine. U937 cells were treated with pervanadate p60Δ8, and an in vitro kinase assay performed. The sample was subjected to SDS-PAGE and transferred to a PVDF membrane. The radioactive band corresponding to pp37 was excised, and phosphoamino acid analysis was performed. The thin layer electrophoresis plate was exposed to PhosphorImager, and the migration of phosphoamino acid standards was visualized by ninhydrin. (C) The amino acid sequence of the p60 cytoplasmic domain that differs between p60Δ8 and p60Δ12 is shown.

bind p60TRAK in the absence of TNF [12]. U937 cells were treated with various concentrations of pervanadate for 30 min, and the cell lysates prepared and affinity precipitated with either GST, p60Δ8, or p60Δ12 followed by in vitro kinase assays. Results in Fig. 2A show that increasing concentrations of pervanadate increased the phosphorylation of p60Δ8, which appeared as a slow migrating band at 37 kDa (pp37). Interestingly, pervanadate had no effect on the phosphoryla-

tion of p60Δ12. The phosphoamino acid analysis of pp37, the phosphorylated p60Δ8, revealed that phosphorylation occurred only on tyrosine (Fig. 2B). p60Δ8 contains a single tyrosine residue at position 331 (Y^{33}) that is not present in p60Δ12. That pervanadate induced the tyrosine phosphorylation of p60Δ8 and not of p60Δ12 suggests that phosphorylation occurred at tyrosine-331 (Fig. 2C). If so, this would explain why pp37 was observed only in precipitates of p60Δ8, but not in that of p60Δ12 (Fig. 2A). Our results thus far suggest that inhibition of PTPase by pervanadate causes a protein tyrosine kinase (PTK), to bind and phosphorylate p60Δ8.

3.3. *Site-specific mutagenesis indicates that pervanadate induces phosphorylation of Tyr^{331}*

To further confirm the site that undergoes phosphorylation by pervanadate, we replaced Tyr^{331} of p60Δ8 with phenylalanine by site-specific mutagenesis. We also made two additional mutants; in one case we replaced Thr^{329} with alanine, and in the other we made a double mutant that contained substitutions at both Thr^{329} and Tyr^{331} (Fig. 2C). Thr 329 was replaced with alanine to determine if the PTK involved is a dual-specificity kinase that can phosphorylate at both tyrosine and threonine residues. The three mutant fusion proteins and wild-type p60Δ8 were used in affinity precipitations of cell lysates from control and pervanadate-treated cells followed by in vitro kinase assays. The tyrosine-phosphorylated form of p60Δ8 (pp37) did not appear in pervanadate-treated cell extracts that had been affinity precipitated with $p60\Delta8^{Y331F}$ or $p60\Delta8^{T329A/Y331F}$ (Fig. 3, lanes 6 and 8), but it appeared in those extracts precipitated with either p60Δ8 (Fig. 3, lane 2) or $p60\Delta8^{T329A}$ (Fig. 3, lane 4). Thus, these results further confirm that Tyr^{331} is the phosphoacceptor site for a PTK

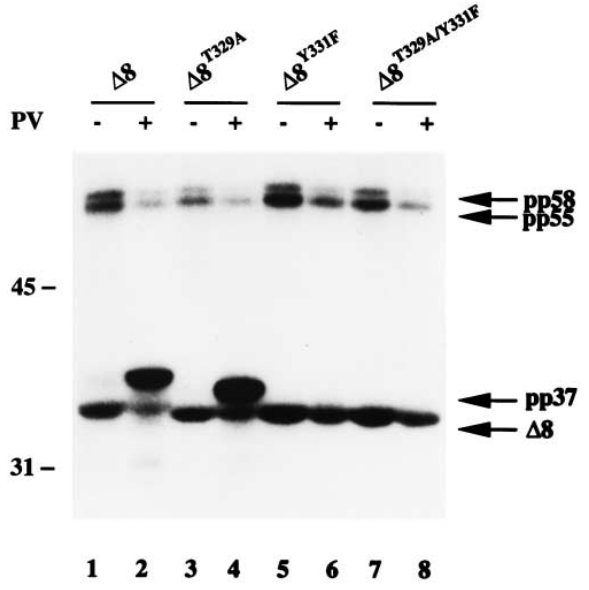

Fig. 3. Site-specific mutagenesis implicates phosphorylation of Tyr^{331} of the p60 TNF receptor. U937 cells were incubated in the presence or absence of pervanadate (100 μM, 30 min, 37°C). Cell lysates were prepared, and affinity precipitations were performed with the indicated wild-type or mutant GST fusion proteins. In vitro kinase assays were performed, the samples were analyzed by 8.5% SDS-PAGE, and the dried gel exposed to X-ray film for 3 h at −70°C. Relative mobilities for various molecules are indicated by the arrows.

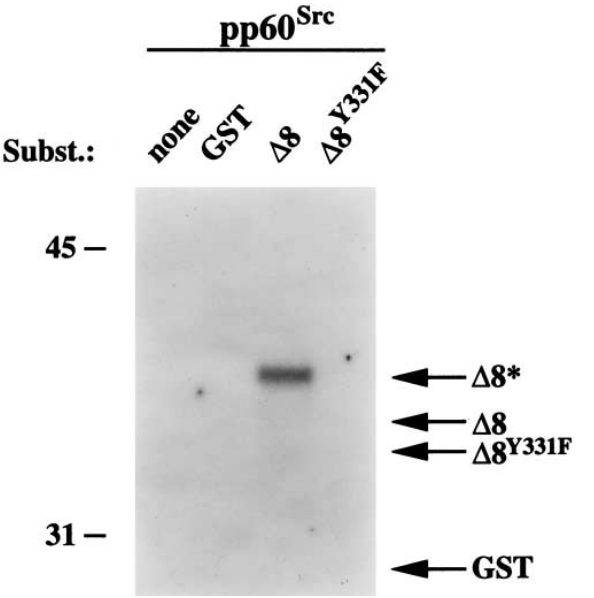

Fig. 4. Tyr^{331} of the p60 cytoplasmic domain is phosphorylated by $pp60^{Src}$ in vitro. Approximately 10 μg of GST, p60Δ8, and $p60\Delta8^{Y331F}$ were first washed three times with kinase buffer. $pp60^{Src}$ (2.5 ng) was mixed with the indicated fusion proteins, and in vitro kinase assays performed in a volume of 20 μl containing 75 mM HEPES pH 8.0, 15 mM $MgCl_2$, 0.2 mM ATP and 10 μCi [γ-^{32}P]ATP for 30 min at 30°C. The reactions were stopped by addition of SDS-sample buffer, and the mixtures boiled and subjected to 9% SDS-PAGE. The dried gel was exposed to X-ray film for 20 h at −70°C. The arrows represent the relative mobility of each fusion protein, and the asterisk indicates the tyrosine phosphorylated form of p60Δ8.

that binds to the CD. Moreover, these results also suggest that this PTK is not likely to be a dual-specificity kinase.

3.4. *$pp60^{Src}$ phosphorylates Tyr^{331} of the p60 cytoplasmic domain in vitro*

To determine the potential PTK responsible for the phosphorylation of the p60CD in pervanadate-treated cells, we tested the ability of several known PTKs to phosphorylate Tyr^{331} in vitro. Of those tested ($pp60^{Src}$, Csk, FGFr, Abl and YES), only $pp60^{Src}$ phosphorylated Tyr^{331} (Fig. 4). $pp60^{Src}$ did not phosphorylate GST nor $p60\Delta8^{Y331F}$.

3.5. *Pervanadate inhibits p60TRAK-mediated phosphorylation of pp55 and pp58*

We have previously shown that p60TRAK, which associates with the TNF receptor, phosphorylates two proteins of approximately 55 and 58 kDa (pp55 and pp58). These proteins were not the endogenous receptor as indicated by western blot with anti-p60 receptor antibody. Quantitation of the experiment described in Fig. 2A revealed that the phosphorylation of pp58 and pp55 decreased with increasing dose of pervanadate, thus indicating that inhibition of a PTPase leads to the inhibition of p60TRAK activity. These results are consistent with that described in Fig. 3 (compare lanes 1, 3, 5 and 7 with 2, 4, 6, and 8).

3.6. *Okadaic acid stimulates p60TRAK-mediated phosphorylation of pp55 and pp58*

We also compared the effect of pervanadate (PTPase inhibitor) with okadaic acid, a specific inhibitor of serine/threonine

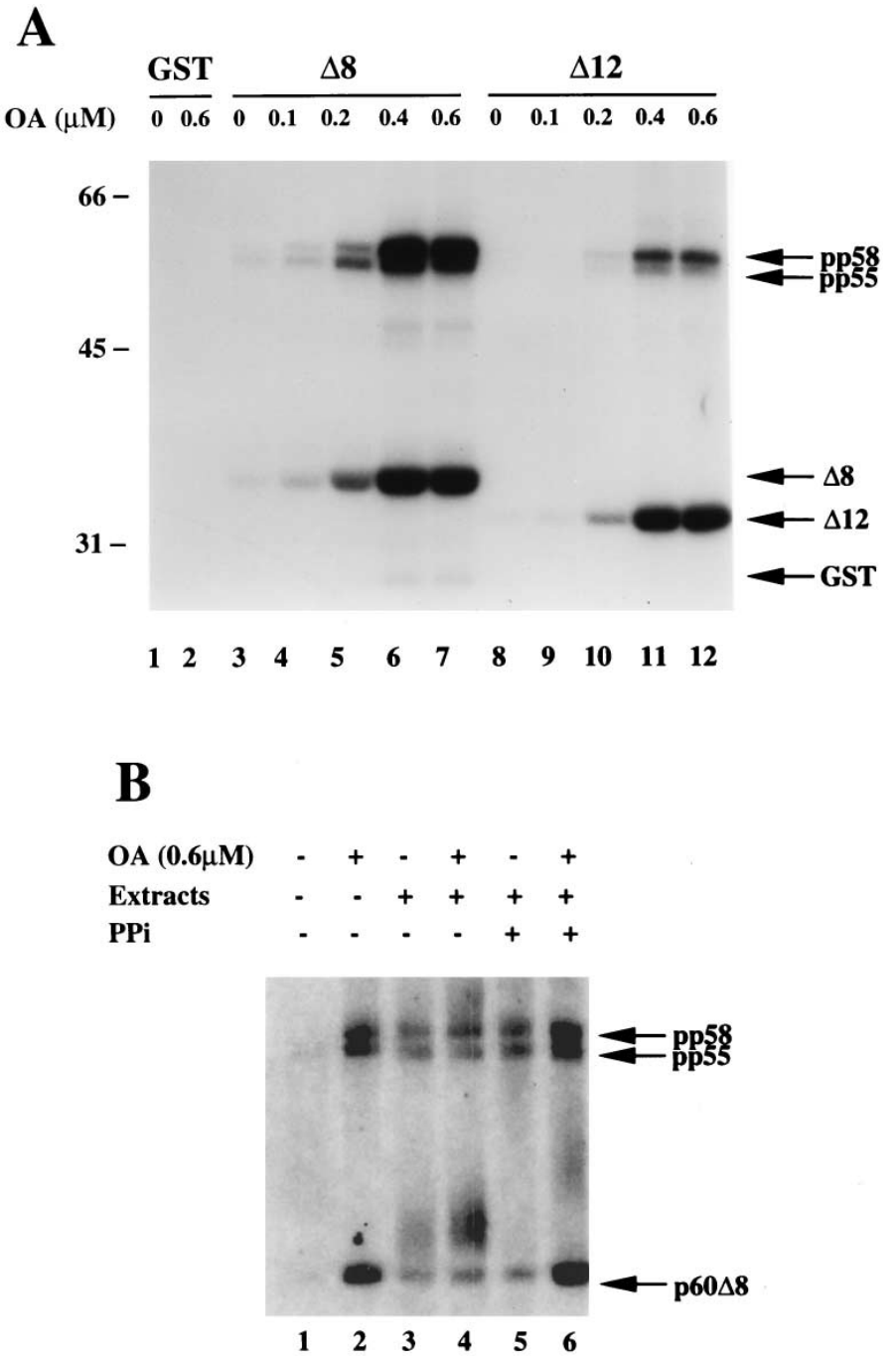

Fig. 5. Okadaic acid increases the activity of p60TRAK. (A) Okadaic acid treatment of cells increases p60TRAK activity. U937 cells were incubated with the indicated concentration of okadaic acid for 30 min at 37°C. Cell lysates were prepared and affinity precipitated, and in vitro kinase assays performed using GST, p60Δ8 and p60Δ12. The molecular mass standards are indicated in kDa. (B) Stimulation of p60TRAK activity by okadaic acid is reversible. U937 cells were incubated in the absence or presence of 0.6 μM okadaic acid for 30 min at 37°C. Cell lysates were prepared and used for affinity precipitation of p60TRAK by p60Δ8. These precipitates were washed first with lysis buffer and then with the phosphatase buffer (50 mM HEPES, pH 7.4, 50 mM NaCl, 1 mM dithiothreitol). In separate tubes, a source of cytosolic protein phosphatases was prepared from 5×10^6 U937 cells lysed in 600 μl in the presence or absence of protein phosphatase inhibitors (10 mM NaF, 2 mM orthovanadate, 0.6 μM okadaic acid) (PPi). Phosphatase assays were performed by the addition of 10 μl of these extracts with the precipitated p60TRAK as indicated and incubated at room temperature for 20 min. These mixtures were then washed sequentially in lysis buffer and kinase buffer. In vitro kinases assays were then performed and the samples were analyzed by 8.5% SDS-PAGE and quantitated on a PhosphorImager.

phosphatase type 2A and type 1 [19], on p60TRAK activity. Okadaic acid is also known to mimic some of the effects of TNF [4,20]. U937 cells were treated with increasing concentrations of okadaic acid for 30 min, and then affinity precipitations of cell lysates were performed with either GST, p60Δ8, or p60Δ12 followed by in vitro kinase assays. In contrast to pervanadate, okadaic acid treatment caused a dose-dependent increase in phosphorylation of p60Δ8, p60Δ12, and the two associated proteins pp58 and pp55 (Fig. 5A). This increase was not due to a direct effect of okadaic acid on

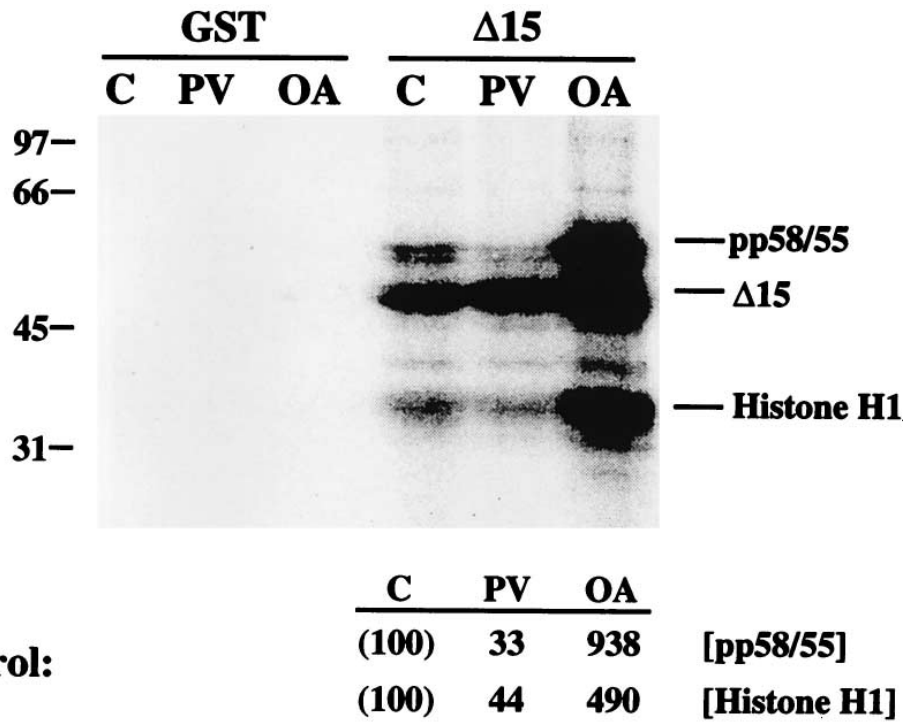

Fig. 6. Histone H1 phosphorylation by p60TRAK is also regulated by phosphatase inhibitors. U937 cells were left untreated (C) or treated with pervanadate (PV) (100 μM, 30 min, 37°C) or okadaic acid (OA) (600 nM, 30 min, 37°C). Cells lysates were prepared and affinity precipitated with GST or p60Δ15 (Δ15), and in vitro kinase assays performed with 5 μg histone H1. The phosphorylation of histone H1 and pp58/pp55 were quantitated by a Phosphorimager and ImageQuant software and presented as percent of control. Similar results were obtained in three independent experiments.

p60TRAK because its addition in the kinase assay had no effect (data not shown).

3.7. *Okadaic acid and pervanadate modulate p60TRAK by phosphorylation*

The activation of p60TRAK by okadaic acid could be due to an increase in either p60TRAK binding to the p60CD or in serine/threonine phosphorylation of p60TRAK. To distinguish between these two possibilities, we isolated p60TRAK from untreated or okadaic acid-treated cells and then assayed for its activity before and after exposure to cellular PPase. Okadaic acid-induced activation of p60TRAK activity (i.e., Fig. 5B, compare lanes 1 and 2) could be reversed to control level by cellular PPase (compare lanes 3 and 4), and the inhibition of the latter by phosphatase inhibitor (PPi) reversed the effect of okadaic acid (compare lanes 2 and 6). The quantitation of lanes 1 and 2 indicates that OA increases phosphorylation of p60Δ8 and pp58/55 by 8.4-folds and 5.5-folds, respectively. Similarly, quantitation of lanes 5 and 6 indicates that OA increases phosphorylation of p60Δ8 and pp58/55 by 3.4-folds and 1.5-folds, respectively. Although cell extracts (cellular PPase) increase the overall phosphorylation but OA has no additional effect (3.1 vs. 3.6 for p60Δ8 and 3.4 vs. 3.7 for pp58/55). Why degree of phosphorylation of p60Δ8 is different from that of pp55/58, is not clear. The difference may lie in the number of phosphorylation sites present in each. Taken together, these results suggest that okadaic acid treatment increased p60TRAK activity not by increasing its binding to p60CD but by enhancing its phosphorylation at serine/threonine.

Although mutation of Tyr^{331} altered its phosphorylation by the associated PTK, none of the mutations affected binding of p60TRAK to p60CD since the extent of its phosphorylation was similar (Fig. 3, compare lanes 1, 3, 5, and 7 for p60Δ8). To further determine that it is not the binding but the pervanadate treatment that inactivates p60TRAK, we examined the phosphorylation of histone H1 by p60TRAK from untreated, pervanadate- or okadaic acid-treated cells. Since histone H1 exhibits the same mobility as p60Δ8 on SDS-polyacrylamide gels, we used p60Δ15 (residues 243–397), which migrates at ~50 kDa for the affinity precipitations. Previous experiments showed no difference in the ability of p60Δ8 or p60Δ15 to precipitate p60TRAK [12]. Quantitation of histone H1 and of pp58/55 revealed that pervanadate inhibited their phosphorylation by 44% and 33%, respectively, and okadaic acid stimulated it by 490% and 940%, respectively (Fig. 6). Thus, p60TRAK appears to be inhibited by tyrosine phosphorylation and activated by serine/threonine phosphorylation.

4. Discussion

The results presented in this report demonstrate that inhibition of PTPases by pervanadate causes the phosphorylation of the tyrosine residue at position 331 in the cytoplasmic domain of the p60 TNF receptor. Furthermore, we show that p60TRAK is inactivated by pervanadate and stimulated by okadaic acid. This is the first report to show tyrosine phosphorylation of the TNF receptor. The cytoplasmic domain of the p60 TNF receptor contains seven tyrosine residues, whereas that of the p80 form of the receptor contains none. Of the seven tyrosine residues in the p60CD, only two (position 331 and 372) are located in the death domain of the

receptor, the region responsible for recruitment of proteins involved in TNF signalling, including cytotoxicity and NF-κB activation (for references see [21]).

Our results indicate that inhibition of PTPase leads to phosphorylation of Tyr^{331} and to inhibition of TNF cell signalling as reported earlier [14–17]. How Tyr^{331} phosphorylation might lead to inhibition of cell signalling is not clear. It is possible, however, that Tyr^{331} phosphorylation affects the recruitment by the receptor of the TNF receptor associated death domain (TRADD) which is necessary for NF-κB activation and growth modulation [21]. This possibility is similar to cellular signalling by other growth factors where association and dissociation of signal-transducing proteins are regulated by tyrosine phosphorylation and dephosphorylation ([22] and references therein).

Our results also suggest that tyrosine phosphorylation leads to a decrease and serine/threonine phosphorylation to an increases in the activity of p60TRAK. This is a situation analogous to cyclin-dependent kinase, whose activity decreases by specific tyrosine phosphorylation and increases by serine/threonine phosphorylation ([23] and other references therein). The type of PTK involved in phosphorylation of the p60 TNF receptor is not clear. Our results, however, show that among several PTKs tested, $pp60^{Src}$ induces optimal phosphorylation of Tyr^{331}. These results are consistent with our previous observations that overexpression of cells with $pp60^{Src}$ leads to resistance to the cytotoxic effects of TNF [24]. Therefore, it is possible that $pp60^{Src}$ is activated on inhibition of PTPase, thus leading to TNF receptor phosphorylation and resistance to ligand-induced cellular responses. Overall our results presented here show that inhibition of PTPase alters the phosphorylation state of the p60 receptor and modulates the receptor-associated kinase. These changes may play a role in TNF-induced NF-κB activation and growth modulation.

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References

[1] Saklatvala, J., Kaur, P. and Guesdon, F. (1991) Biochem. J. 277, 635–642.
[2] Donato, N.J., Gallick, G.E., Steck, P.A. and Rosenblum, M.G. (1989) J. Biol. Chem. 264, 20474–20481.
[3] Marino, M.W., Feld, L.J., Jaffe, E.A., Pfeffer, L.M., Han, H.-M. and Donner, D.B. (1991) J. Biol. Chem. 266, 2685–2688.
[4] Brown, K., Gerstberger, S., Carlson, L., Franzoso, G. and Sienbenlist, U. (1995) Science 267, 1485–1491.
[5] Kanety, H., Feinstein, R., Papa, M.Z., Hemi, R. and Karasik, A. (1995) J. Biol. Chem. 270, 23780–23784.
[6] Guo, D. and Donner, D.B. (1996) J. Biol. Chem. 271, 615–618.
[7] Hotamisligil, G.S., Peraldi, P., Budavari, A., Ellis, R., White, M.F. and Spiegelman, B.M. (1996) Science 271, 665–670.
[8] Aggarwal, B.B. and Natarajan, K. (1996) Eur. Cytokine Network 71, 93–124.
[9] Vandenabeele, P., Declercq, W., Beyaert, R. and Fiers, W. (1995) Trends Cell Biol. 5, 392–399.
[10] Darnay, B.G., Reddy, S.A.G. and Aggarwal, B.B. (1994) J. Biol. Chem. 269, 20299–20304.
[11] Darnay, B.G., Reddy, S.A.G. and Aggarwal, B.B. (1994) J. Biol. Chem. 269, 196787–196790.
[12] Darnay, B.G., Singh, S., Chaturvedi, M.M. and Aggarwal, B.B. (1995) J. Biol. Chem. 270, 14867–14870.
[13] Totpal, K., Aggarwal, S. and Aggarwal, B.B. (1992) Cancer Res. 52, 2557–2562.
[14] Menon, S.D., Guy, G.R. and Tan, Y.H. (1995) J. Biol. Chem. 270, 18881–18887.
[15] Singh, S. and Aggarwal, B.B. (1995) J. Biol. Chem. 270, 10631–10639.
[16] Singh, S., Darnay, B.G. and Aggarwal, B.B. (1996) J. Biol. Chem. 271, 31049–31054.
[17] Totpal, K., LaPushin, R., Kohno, T., Darnay, B.G. and Aggarwal, B.B. (1994) J. Immunol. 153, 2248–2257.
[18] Chainy, G.B.N., Singh, S., Raju, U. and Aggarwal, B.B. (1996) J. Immunol. 157, 2410–2417.
[19] Goris, J., Hermann, J., Hendrix, P., Ozon, R. and Merlevede, W. (1989) in: Advances in Protein Phosphatases (Merlevede, W. and Di Salvo, J., Eds.) vol. 5, pp. 579–592, Leuven University Press, Leuven, Belgium.
[20] Guy, G.R., Cao, X., Chua, S.P. and Tan, Y.H. (1992) J. Biol. Chem. 267, 1846–1852.
[21] Darnay, B.G. and Aggarwal, B.B., J. Leukoc. Biol. (in press).
[22] Ullrich, A. and Schlessinger, J. (1990) Cell 61, 203–212.
[23] Morgan, D.O. (1995) Nature 374, 131–134.
[24] Aggarwal, B.B., Totpal, K., Ali-Osman, F., Budde, R. and Pocsik, E. (1994) FEBS Lett. 345, 219–224.