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Analysis of trk A and p53 association

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Abstract trk A tyrosine kinase (the high affinity receptor for nerve growth factor) binds to the p53 tumour suppressor protein in vitro and in vivo. Our aim was to determine which regions of p53 are involved in trk A association. In vitro binding experiments using baculovirus expressed trk A and in vitro transcribed and translated C-terminus p53 deletion mutants show amino acids 327–338 critical for association. Also, analysis with mutants at the N-terminus, conserved regions II, III, IV and V or amino acid positions 173, 175, 181, 248 and 249 (which are amino acids frequently mutated in a variety of neoplasms and transformed cell lines), show that these sites are not involved in trk A binding. Importantly, similar results are obtained after immunoprecipitation of lysates from p53 negative fibroblasts expressing trk A and the above p53 mutant proteins. These data suggest that the amino-terminus of the oligomerisation domain of p53 is involved in p53/trk A association.

Key words: trk A; c-abl; p53 mutant; Association; Mapping

1. Introduction

The p53 tumour suppressor phosphoprotein is characterised by having an N-terminus transcription region, a DNA binding domain and a C-terminus oligomerisation domain [1]. The biochemical activity of p53 required for suppression requires its ability to bind to DNA in a specific manner and to function as a transcription factor [1–3]. Several genes are activated by p53 and their promoters have been found to contain p53 binding sequences (reviewed in [1–4]). Interestingly, the N-terminus region binds to several proteins (or protein complexes) involved in transcription, these include TBP and TFIID [5–7]. Although transcriptional activation represents an important role, p53 also is able to repress transcription from several promoters in a TATA dependent or independent manner [8].

The C-terminus oligomerisation domain is not involved directly in DNA specific recognition; however, there is evidence showing that it contributes to the DNA binding and transcriptional activity of p53 [3,9–14].

p53 is a multifunctional protein involved in apoptosis, differentiation [15,16] and regulation of its own expression (reviewed in [3]). Thus, it binds to proteins, such as MDM-2, CDC2, WT1, RPA, tms-1, the regulatory B-subunit of protein kinase II [1,3] as well as to the high affinity receptor for nerve growth factor (NGF) trk A [16,17].

trk A is a receptor tyrosine kinase which upon NGF stimulation dimerises/oligomerises, becomes phosphorylated on tyrosine and activated to initiate a signaling cascade [18]. Activated trk A can in turn bind and/or phosphorylate molecules such as PLC-γ1, shc, SNT and MAP kinases [19,20] PI-3 kinase does not appear to bind directly to trk A, but most likely through shc, since mutation of the shc binding site affects the level of activation of PI-3 kinase by NGF in PC12 cells [21,22].

Transfection of a p53 murine temperature sensitive mutant (val-135) (ts p53) [23] into PC12 cells overexpressing trk A [24], induces morphological changes and trk A tyrosine hyperphosphorylation when cells are grown at 32°C (p53 expressed in the wild type configuration), but not at 37°C (p53 expressed in the mutant configuration) in the absence of NGF stimulation. Interestingly, in Saos-2 cells (an osteosarcoma cell line devoid of trk A and p53 expression [25]) transfected with trk A, trk A phosphorylation is not detected when expressed on its own in cells grown at either temperature; however, phosphorylation is seen when co-transfected with ts p53 and cells grown at 32°C in the absence of NGF stimulation. To determine a possible mechanism by which p53 and trk A induce a differentiative response, association of these proteins was analysed. Affinity purified wild type p53 and trk A associate in vitro. Association is also detected in vivo in PC12 and NIH3T3 cells overexpressing trk A and ts p53, PC12 and NIH3T3 expressing trk A alone and in non-transfected PC12 cells, indicating that overexpressed and endogenous levels of trk A and p53 can bind. These experiments suggest that p53 has a novel function which involves the stimulation of signalling pathways possibly through the association and hyperphosphorylation of trk A [16].

Recently we have shown that the c-abl non-receptor tyrosine kinase (reviewed in [26]) is involved in p53 and trk A binding. Endogenous c-abl can be detected when p53 and trk A are co-precipitated. Association of p53/c-abl/trk A is observed in NIH3T3 and PC12 cells transfected with trk A and ts p53, NIH3T3 and PC12 cells transfected with trk A alone and in untransfected PC12 cells suggesting that endogenous and overexpressed p53, trk A and c-abl can associate. Importantly, binding of p53 to trk A is not detected in NGF stimulated abl negative fibroblasts expressing transfected trk A and endogenous p53. Similar results are obtained when in these cells p53 is activated and its levels increased by exposure to γ radiation; suggesting that p53 and trk A preferentially associate in the presence of c-abl [17]. To further our inves-

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tigation we wanted to determine which domains of p53 are involved in trk A binding.

2. Materials and methods

2.1. Cell culture and transfection

p53−/− (obtained from mice with disrupted p53 alleles) and p53−/− transfected with trk A (p53−/− trk A) fibroblasts were maintained in Dulbecco's Modified Eagle's medium supplemented with 10% heat inactivated foetal bovine serum (Gibco BRL). Cultures were supplemented with 2 mM glutamine, 1 mM sodium pyruvate, and 100 IU/ml penicillin/streptomycin (Gibco BRL).

p53−/− cells were co-transfected with pMextrk (a plasmid coding for human proto-trk A) and pSV2neo using Lipofectamine as described by the manufacturers (Gibco BRL). 1×10^5 cells were plated in 10 cm plates and incubated overnight with media as described above. The cells were washed three times with serum free medium and DNA:Lipofectamine mix (15 μg:30 μl) was added to cells in 3 ml of serum free medium and left to incubate for 4 h at 37°C, washed and incubated for another 48 h in medium supplemented with serum. Then colonies were selected in G418 (600 μg/ml) at 37°C and resistant colonies were screened for trk A expression by immunoprecipitation with anti-trk A B-3 antibodies (Santa Cruz Biotechnology). p53−/− trk A cells were maintained in 10 mg/ml G418 and passaged at 70–80% confluency.

For transient transfections 1×10^8 p53−/− or p53−/− trk A cells were mixed with 20 μg of each p53 mutant (amount previously titrated to express similar amounts of protein) and subjected to electroporation. Cells were grown at 37°C for 48 h and subjected to lysis.

Sf9 cells were grown in Grace's medium supplemented with 10% foetal calf serum. 10^7 cells were infected with baculovirus expressing proto-trk A at a multiplicity of 10 and cells were lysed 48 h post-infection.

2.2. p53 mutants

These include deletion mutants in conserved regions I–V (p53ΔI 13–19, p53ΔII 117–142, p53ΔIII 171–181, p53ΔIV 234–258 and p53ΔV 270–286) [27], three N-terminal mutants p53NΔ 18 (Midgley and Lane, personal communication), p53Δ 28–40 and p53Δ 41–49 (p53Δ 28–40 and p53Δ 41–49 were made as described in [27]), six C-terminus mutants p53Δ 370, p53CΔ 30, p53Δ 347, p53Δ 338, p53Δ 327 and p53Δ 291 [28–30] and a series of point mutants (p53 173L; p53 175H; p53 175P; p53 181L; p53 248W and p53 249S) [31].

p53 mutants cloned into pGEM4Z and pGEM3Z were used for in vitro transcription and translation. Mutants cloned into pJ3Ω, pJ4Ω, pCMV and pCDNA 3.1 (Invitrogen) were used for eukaryotic expression after transfection. The *Eco*RI/*Bam*HI fragment from p53Δ 28–40 or p53Δ 41–49 and the *Xba*I/*Hin*dIII fragment from NΔ 18 were subcloned into pCDNA 3.1 using standard techniques.

2.3. Immunoprecipitation and Western blotting

For in vitro experiments SF9 cells were lysed in ice cold 20 mM Tris–HCl pH 8, 137 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.15 U/ml aprotinin, 20 μM leupeptin, 1 mM Na_3VO_4 and 1% NP-40 (lysis buffer). Lysates with similar protein concentration were incubated with rabbit anti-trk 203 antibodies for 1 h at 4°C, precipitates were collected with 30 μl of protein G Sepharose for 1 h 30 min at 4°C and washed three times with ice cold lysis buffer. These were incubated further with equal amounts of in vitro transcribed/translated p53 mutants using the TNT T7 or SP6 Quick coupled transcription translation System (following manufacturer's instructions; Promega) for 1 h at 4°C and washed three times with lysis buffer. The pellets were boiled for 2 min in sample buffer containing 10% glycerol, 2% sodium dodecyl sulphate (SDS), 0.1 mM dithiothreitol and 0.001% bromophenol blue. Samples were run on 7.5% SDS polyacrylamide gels. The resolved proteins were transferred onto nitrocellulose. The blot was blocked for 1 h in 137 mM NaCl and 20 mM Tris–HCl pH 8 (TBS) containing 2% bovine serum albumin (BSA); incubated with sheep anti-p53 polyclonal antibodies at 4°C overnight and washed three times with TBS containing 0.2% Tween 20 (TBS-T) for 5 min at room temperature. Bound antibody was detected by incubating with peroxidase conjugated donkey anti-sheep antibodies (diluted 1:20 000 in TBS-T) for 1 h at room temperature, washed three times with TBS-T and visualised by chemoluminescence (ECL Amersham).

Blots were stripped of antibody by incubating with 2% SDS, 100 mM 2-mercaptoethanol, 62.5 mM Tris–HCl pH 6.8 for 1 h at 70°C, rinsed three times for 15 min at room temperature with TBS and then probed again with anti-trk 203 antibodies and developed using horse radish peroxidase conjugated swine anti-rabbit antibodies.

For in vivo experiments cells were treated for 5 min with 100 ng/ml NGF when indicated, then were rinsed three times with ice cold TBS and lysed in 100 μl of ice cold lysis buffer. The protein concentration was determined using the BioRad protein assay. Lysates were immunoprecipitated with DO-1, PAb 421 or anti-trk A 203 antibodies for 2 h at 4°C, precipitates were collected with 35 μl of Protein G Sepharose for 1 h at 4°C and washed three times with ice cold lysis buffer. Pellets were boiled in sample buffer. Samples were resolved on 7.5% SDS–polyacrylamide gels and the proteins transferred onto nitrocellulose. Blots were incubated with sheep anti-p53 or anti-trk 203, diluted in TBS-T, overnight at 4°C and then washed. Bound antibody was detected by incubation with peroxidase-conjugated anti-sheep or anti-rabbit antibodies (Dako) at a dilution of 1:20 000 in TBS-T, for 1 h at room temperature and followed by three washes with TBS-T. Proteins were visualised by using enhanced chemiluminescence (ECL Amersham).

3. Results and discussion

3.1. In vitro analysis of p53 and trk A association

Human p53 mutants used in this study have been described previously [27–31]. These include deletion mutants in conserved regions I–V (p53ΔI 13–19, p53ΔII 117–142, p53ΔIII 171–181, p53 ΔIV 234–258 and p53ΔV 270–286); three N-terminal mutants with deletions in the transactivation region

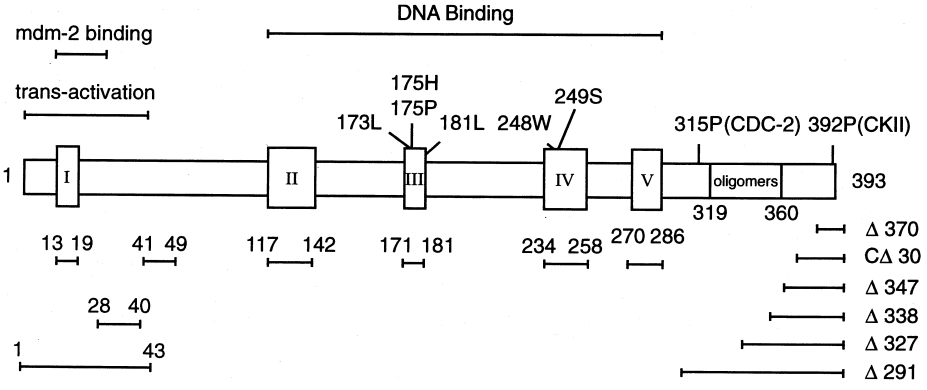

Fig. 1. p53 protein diagram showing the evolutionally conserved regions I–V, the sites for phosphorylation by CDC2 and CKII, as well as the transactivation region, DNA and MDM-2 binding domains. Deletion mutants at the N-terminus, C-terminus and conserved regions are shown below and point mutants are shown above.

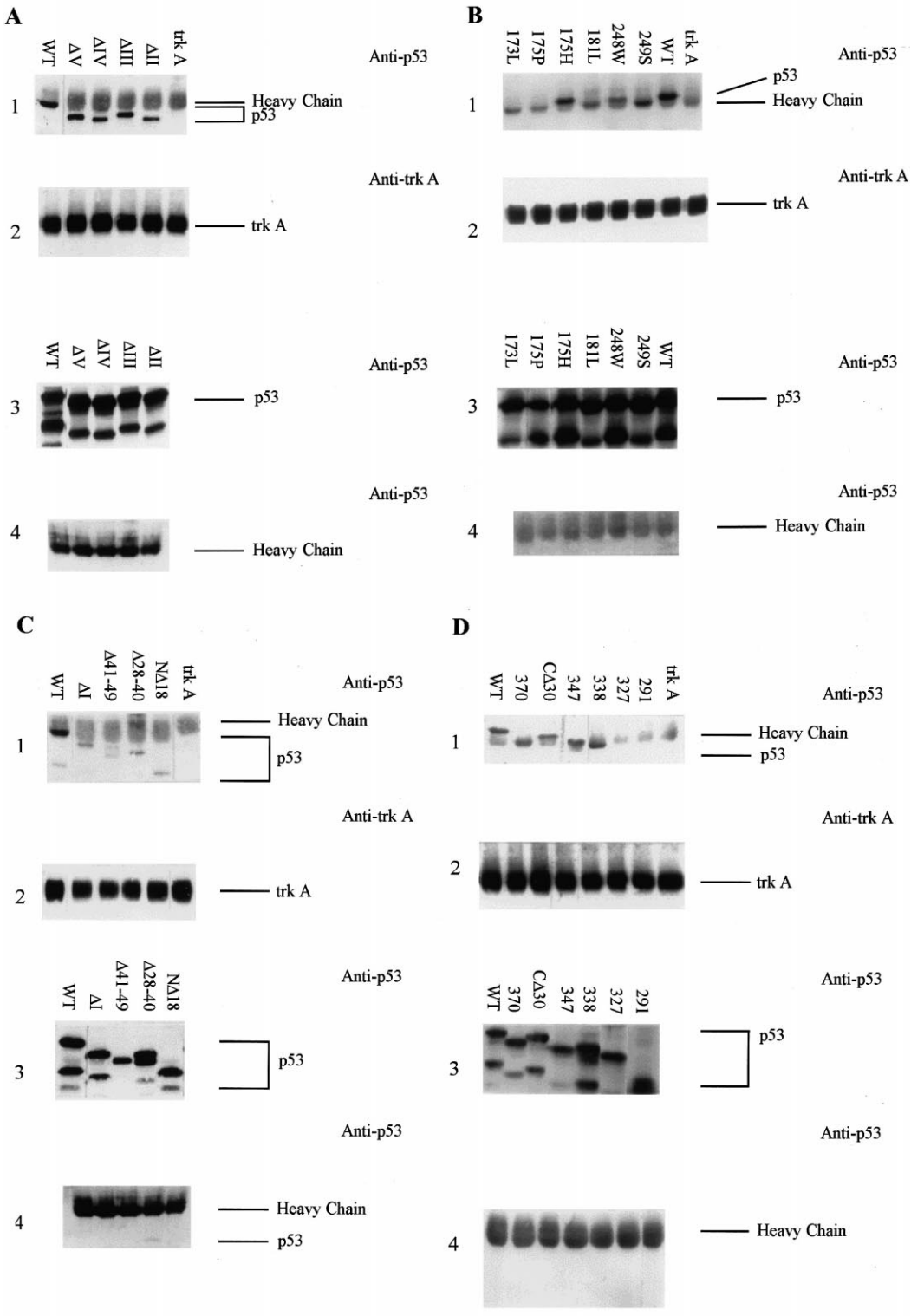

Fig. 2. In vitro association of (A) p53 conserved region deletion mutants II–V; (B) p53 point mutants 173L, 175P, 175H, 181L, 248W and 249S; (C) p53 N-terminus deletion mutants; (D) p53 C-terminus deletion mutants with trk A. (1) Similar amounts of transcribed/translated p53 mutants were incubated with immunoprecipitated trk A. Complexes were subjected to immunoblotting and the presence of p53 probed with sheep anti-p53 antibodies. Each lane shows binding of mixtures containing similar amounts of trk A and each p53 mutant protein. (2) Stripped blot probed with rabbit anti-trk 203 antibodies to show the presence of trk A. (3) Panel of in vitro transcribed/translated p53 mutant proteins prior to immunoprecipitation showing similar levels of expression used in this study. (4) p53 proteins immunoprecipitated with rabbit anti-trk 203 antibodies to detect non-specific binding. Blots probed for the presence of p53 with sheep anti-p53 antibodies. This is a representative of several experiments.

from which amino acids 1–43 (p53 NΔ 18) (Midgley and Lane, personal communication), 28–40 (p53Δ 28–40) and 41–49 (p53Δ 41–49) have been removed (p53Δ 28–40 and p53Δ 41–49 were made as described in [27]); six termination C-terminus mutants from which amino acids 370–393 (p53Δ 370), 363–393 (p53CΔ 30), 347–393 (p53Δ 347), 338–393 (p53Δ 338), 327–393 (p53Δ 327) and 291–393 (p53Δ 291) have been deleted and a series of point mutants previously identified in anogenital neoplasms and transformed cell lines (p53 173L; p53 175H; p53 175P; p53 181L; p53 248W and p53 249S) (Fig. 1).

Initially the ability of p53 mutant proteins to associate with trk A was assayed by in vitro binding experiments. Cell extracts of Sf9 cells infected with baculovirus expressing human proto-trk A [32] were immunoprecipitated with anti-trk 203 antibodies which recognise the last 15 amino acids of trk A [33]. Fig. 2(A2–D2) show similar amounts of trk A in all experiments (in agreement with results obtained by other laboratories, baculovirus expressed trk A can be seen as a single fast migrating band [32]). Then, the immunoprecipitates were incubated with similar amounts of in vitro transcribed and translated wild type or mutant p53 (Fig. 2, A3 to D3) and washed extensively. Bound proteins were analysed by Western blotting; the presence of p53 was determined by incubating with sheep anti-p53 antibodies and the presence of trk A was detected with rabbit anti-trk 203 antibodies.

Fig. 2, A1 shows that wild type and deleted p53 at conserved regions II–V can co-precipitate with trk A. Also, all p53 point mutants tested bind to immunoprecipitated trk A but with different abilities (Fig. 2, B1). Importantly, these mutant proteins are not immunoprecipitated by rabbit anti-trk 203 antibodies as shown by immunoblotting with sheep anti-p53 antibodies (Fig. 2, A4 and B4). Binding is also detected when two C-terminal phosphorylation mutants with point substitutions at serine residues phosphorylated by CDC2 (p53Ala 315) and CKII (p53 Ala 392) are used (data not shown). These results suggest that conserved regions II–V and point mutants located in the DNA binding domain are not involved in p53/trk A association.

Binding experiments of trk A and the p53 N-terminal p53Δ 28–40, p53Δ 41–49, p53NΔ 18 and p53Δ I mutant proteins show all mutants able to co-precipitate with trk A. However, when compared to wild type p53, they have reduced binding ability only detected in long exposures (Fig. 2, C1). Immunoprecipitation of in vitro transcribed and translated proteins with anti-trk 203 antibodies followed by immunoblotting with sheep anti-p53 antibodies shows low levels of cross-reactivity with p53NΔ 18 (a truncation of the first 43 amino acids), similar to those detected by co-precipitation (Fig. 2, C4); suggesting that in vitro binding between this mutant and trk A probably does not take place.

During experiments with the p53 C-terminal deleted proteins it can be seen that trk A binds to p53CΔ 30, p53Δ 370, p53Δ 347 and p53Δ 338, but does not appear to associate with the extensive deletions p53Δ 327 and p53Δ 291 (Fig. 2, D1). Also, anti-trk 203 antibodies do not immunoprecipitate these proteins as shown by immunoblotting with sheep anti-p53 antibodies (Fig. 2, D4). This result suggests that a possible binding site is located between amino acids 327 and 338 which corresponds to the amino terminus of the oligomerisation domain of p53.

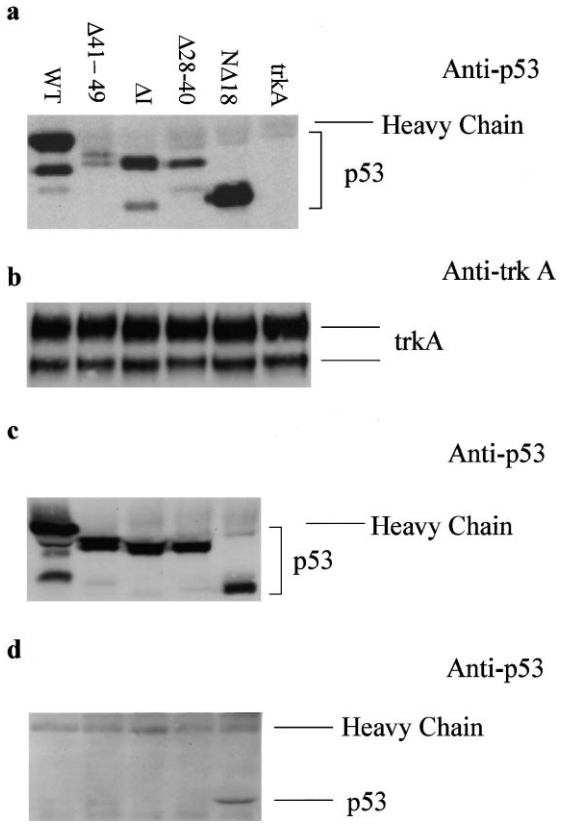

Fig. 3. In vivo association of p53 N-terminus mutants and trk A. a: Similar amounts of lysates of p53−/− trk A cells transiently transfected with wild type p53, p53Δ 41–49, p53Δ I, p53Δ 28–40 and p53NΔ 18 and stimulated with NGF were immunoprecipitated with anti-trk A 203 and the presence of p53 was determined by immunoblotting with sheep anti-p53 antibodies. b: Stripped blot incubated with rabbit anti-trk 203 antibodies to show similar amounts of trk A. c: Similar amounts of lysates of p53−/− trk A cells transiently transfected with wild type p53, p53Δ 41–49, p53Δ I, p53Δ 28–40 and p53NΔ 18 and stimulated with NGF were immunoprecipitated with PAb 421 antibodies. The presence of similar amounts of p53 was determined by immunoblotting with sheep anti-p53 antibodies. d: Similar amounts of lysates of p53−/− cells transiently transfected with wild type p53, p53Δ 41–49, p53Δ I, p53Δ 28–40 and p53NΔ 18 were immunoprecipitated with anti-trk 203 antibodies and the presence of p53 was determined by immunoblotting with sheep anti-p53 antibodies. For these experiments similar amounts of p53 mutants transiently expressed in p53−/− trk A or p53−/− cells was used. This is a representative of several experiments.

3.2. In vivo analysis of p53 and trk A association

p53 negative fibroblasts (obtained from mice with disrupted p53 alleles) transfected with human proto-trk A (p53−/− trk A) were transiently transfected with the N-terminus (p53Δ 28–40, p53Δ 41–49 and p53NΔ 18) and conserved region I (p53Δ I) deletion mutants. Similar amounts of cell lysates were immunoprecipitated with rabbit anti-trk 203 antibodies and bound proteins analysed by immunoblotting with sheep anti-p53 antibodies. Fig. 3a shows these mutants able to co-precipitate with trk A; however, p53Δ 28–40 and p53Δ 41–49 are more easily detected after long exposures. Similar amounts of transiently expressed mutant proteins used for co-precipitation can be detected in lysates of p53−/− trk A cells immunoprecipitated with PAb 421 (a monoclonal antibody that recognises p53 at amino acids 370–378 [34]) followed by immunoblotting with sheep anti-p53 antibodies (Fig. 3c). The presence of the slow and fast migrating forms of trk A can

be observed after incubation with anti-trk 203 antibodies (Fig. 3b). Immunoprecipitation with anti-trk 203 antibodies of cell lysates from p53−/− cells transiently expressing these mutant proteins followed by immunoblotting with sheep anti-p53 antibodies shows a very low level cross-reactivity detected only with p53NΔ 18 (Fig. 3d). However, when comparing Fig. 3b,d it can be seen that the level of co-precipitated p53NΔ 18 is higher than the non-specifically recognised by anti-trk 203 suggesting that, in vivo, this mutant protein binds to trk A.

Immunoprecipitation of p53−/− trk A cell lysates transiently expressing the C-terminus mutant proteins with anti-trk 203 antibodies, followed by immunoblotting with sheep anti-p53 antibodies, shows trk A binding to p53CΔ 30, p53Δ 370, p53Δ 347, p53Δ 338. Importantly, trk A does not appear to associate with p53Δ 291 and p53Δ 327 (Fig. 4a). Immunoprecipitation of these lysates with anti-p53 DO-1 antibodies (a monoclonal antibody that recognises amino acids 20–25 of human p53 [35]) followed by immunoblotting with sheep anti-p53 antibodies shows similar amounts of mutant proteins used for co-precipitation experiments (Fig. 4c). The presence of similar amounts of trk A can be detected after incubation with anti-trk 203 antibodies (Fig. 4b). Immunoprecipitation, with anti-trk 203 antibodies, of lysates from p53−/− cells transiently expressing the C-terminus mutants followed by immunoblotting with sheep anti-p53 antibodies, shows that anti-trk 203 antibodies do not recognise these truncated proteins (Fig. 4d).

In agreement with the in vitro findings, experiments with p53−/− trk A cells transiently co-transfected with the conserved region and point mutants show trk A association with all mutant proteins. Also, p53 173L, p53 175P, p53 181L, p53 248W appear to bind to trk A with different abilities (data not shown).

Overall, data obtained in vivo suggest that p53/trk A binding most likely involves amino acids 327–338 from the amino terminus of the oligomerisation domain of p53.

Results obtained from in vitro binding experiments suggest that amino acids 327–338 of p53 are involved in p53 and trk A association. Co-precipitation experiments with the N-terminus deletion mutants demonstrate that these proteins bind to trk A with low ability. Anti-trk A 203 antibodies appear to non-specifically recognise the product of p53NΔ 18 suggesting that association between this mutant and trk A does not take place.

In vitro results with the conserved region mutants show that these truncated proteins can bind to trk A. Similarly, all point mutants associate with trk A with a range of abilities (p53 175H, p53 181L, p53 248W and p53 249S); suggesting that these sites are not involved in p53 and trk A binding. Unfortunately, results for the association between trk A and p53 173L or p53 175P are not conclusive owing to co-migration of these proteins with the heavy chain of the immunoprecipitating antibody (Fig. 2, B1).

The overall consensus is that in vitro transcribed and translated p53 proteins have 'sticky' properties [36]. Therefore, in vitro results were analysed further by carrying out in vivo experiments. Immunoprecipitations of cell lysates from p53−/− trk A cells, expressing conserved regions II–V or point mutants, with anti-trk A 203 antibodies followed by immunoblotting with sheep anti-p53 antibodies show all able to bind to trk A (data not shown). Thus, suggesting

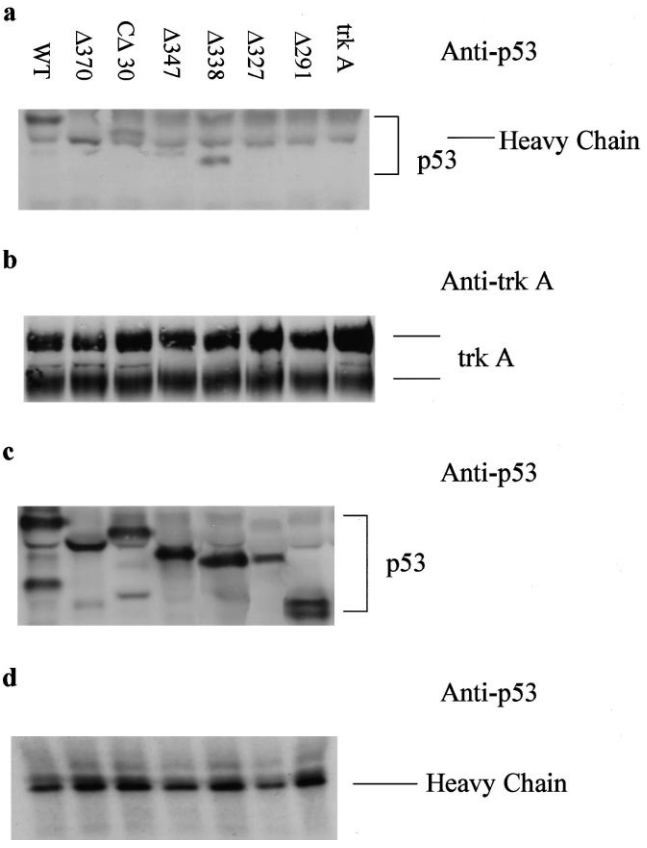

Fig. 4. In vivo association of p53 C-terminus mutants and trk A. a: Similar amounts of lysates of p53−/− trk A cells transiently transfected with wild type p53, p53Δ 370, p53CΔ 30, p53Δ 347, p53Δ 338, p53Δ 327 and p53Δ 291 and stimulated with NGF were immunoprecipitated with anti-trk A 203 and the presence of p53 was determined by immunoblotting with sheep anti-p53 antibodies. b: Stripped blot incubated with rabbit anti-trk 203 antibodies to show similar amounts of trk A. c: Similar amounts of lysates of p53−/− trk A cells transiently transfected with wild type p53, p53Δ 370, p53CΔ 30, p53Δ 347, p53Δ 338, p53Δ 327 and p53Δ 291 and stimulated with NGF were immunoprecipitated with DO-1 antibodies. The presence of similar amounts of p53 was determined by immunoblotting with sheep anti-p53 antibodies. d: Similar amounts of lysates of p53−/− cells transiently transfected with wild type p53, p53Δ 370, p53CΔ 30, p53Δ 347, p53Δ 338, p53Δ 327 and p53Δ 291 were immunoprecipitated with anti-trk 203 antibodies and the presence of p53 was determined by immunoblotting with sheep anti-p53 antibodies. For these experiments similar amounts of p53 mutants transiently expressed in p53−/− trk A or p53−/− cells was used. This is a representative of several experiments.

that these sites are not involved in p53/trk A association. These results agree with those obtained in vitro.

During immunoprecipitation of cell lysates from p53−/− trk A cells, expressing the N-terminus mutants, with anti-trk A 203 antibodies followed by immunoblotting with sheep anti-p53 antibodies p53Δ 28–40, p53Δ 41–49, p53NΔ 18 and p53Δ I are able to co-precipitate with trk A, but in these experiments, p53Δ 28–40 and p53Δ 41–49 appear to bind with low ability.

A comparison of in vitro and in vivo results suggest that variability in binding efficiency by the N-terminus and point mutants, when expressed in different environments, could be due to unknown protein conformational changes acquired as a result of the mutation, rather than to the absence of a site directly involved in trk A binding.

Overall, results obtained with the N-terminus and point mutant proteins indicate that these regions are most likely not involved in p53 and trk A association.

As of results obtained in vitro, in vivo analyses display non-specific recognition of p53NΔ 18 by anti-trk 203 antibodies (observed after direct immunoprecipitation); however, the level of co-precipitated p53NΔ 18 is higher than that non-specifically recognised by anti-trk 203 suggesting that, in vivo, p53NΔ 18 binds to trk A; thus, amino acids 1–43 are not involved in trk A/p53 association.

There is evidence showing that p53Δ I does not bind to MDM-2 [27]. Hence, our results suggest that trk A may not necessarily interfere with MDM-2 binding because p53Δ I can associate with trk A.

In vivo analysis of trk A association with the C-terminus mutant proteins show p53Δ 338, p53Δ 347, p53Δ 370 and p53CΔ 30 able to bind to the receptor; however, p53Δ 291 and p53Δ 327 do not co-precipitate with trk A, suggesting that the binding site is located at amino acids 327–338. This result agrees with data obtained in vitro.

p53 and c-abl can bind after genotoxic damage [37–39]. Also, recently we have shown that c-abl is required for p53 and trk A association [17]. Since p53/trk A binding is preferentially detected in the presence of c-abl; association between p53 mutants and endogenous c-abl was analysed using anti-abl ab-3 antibodies which can co-precipitate p53/c-abl/trk A [17]. However, when anti-abl ab-3 antibodies are used to directly immunoprecipitate wild type and p53 mutant proteins transiently expressed in abl−/− fibroblasts; immunoblotting with anti-p53 antibodies shows that, as already published, anti-abl ab-3 does not recognise wild type p53 but that it can non-specifically recognise several mutant proteins (data not shown), strongly suggesting that the 'sticky' properties generally attributed to mutant p53 proteins are influencing these results [36].

Overall, it can be concluded that in vitro trk A/p53 association appears to require amino acid positions 327–338 located at the amino terminus of the oligomerisation domain of p53. Importantly, the in vivo analysis gave similar results, providing further evidence that this site is most likely involved in p53/trk A binding. Since our previous data show that c-abl is required for this association, it is possible that this region is involved in c-abl and p53 recognition. However, the latter needs to be characterised further.

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