# SHP2 binds catalase and acquires a hydrogen peroxide-resistant phosphatase activity via integrin-signaling

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**Abstract** **Here, we examined whether catalase binds SHP2 and alters SHP2 susceptibility to $H_2O_2$. Our results indicated that serum and fibrinogen commonly evoked catalase binding to SHP2 in HeLa and A549 cells in a herbimycin-A and TNFα sensitive manner. Expression of active catalase nearly 15-fold over control levels in tet-off HeLa cells substantially increased the SHP2 binding, and the catalase-associated SHP2 displayed significantly high phosphatase activities with a $H_2O_2$-resistance compared to those with little catalase. Site-directed mutagenesis at 280 abolished the binding capability of catalase to SHP2–SH2 in vitro. These results suggest that catalase-280pYIQV binds SHP2 via integrin-signaling to increase a $H_2O_2$-resistant SHP2 activity.**



*Keywords:* Catalase; SHP2; Integrin; Hydrogen peroxide; Tet-off HeLa; A549; TNFα

## 1. Introduction

Accumulated evidence indicates that $H_2O_2$ acts as a second messenger of mitogenic signaling [1–3]. In facts, multiple growth factors commonly elicit a transient generation of intracellular $H_2O_2$ [4–7]. Scavengers such as exogenously introduced CAT and *N*-acetylcysteine substantially decrease P-Tyr formation and abort MAPK activation and cell proliferation [4,8,9]. The mechanism by which $H_2O_2$ promotes the signaling still remains unclear, but a parallel formation of P-Tyr with the generation suggests that $H_2O_2$ transiently inhibits PTP [10–13], whereby RPTK-evoked P-Tyr formation sustains to sequester SH2- and PTB-domain-containing molecules [4]. Consistent with this view, all PTP active centers commonly comprise a single sulfhydryl group [14,15] and its oxidation by $H_2O_2$ inactivates them. Thus, mitogenic $H_2O_2$ appears act to suppress PTP activity.

Recent studies, however, demonstrated that a ubiquitous non-transmembrane PTP, SHP2 (PTPN11), positively regulates the mitogenic signaling [16–18] despite of its antagonistic action against RPTK-mediated P-Tyr formation. How can redox-sensitive SHP2 act as a positive regulator during the mitogenic $H_2O_2$ generation? SHP2 comprises one catalytic and two SH2 domains capable of binding Gab-1 [19], SHPS-1 [20–22], EGF-R [16] and PDGF-R [17]. A free form of SHP2 displays little activity due to folding of its N-terminus to the cleft of catalytic domain. Binding of ligands unfolds N-terminus from the catalytic domain resulting activity expression, as deletion of the N-terminus SH2 makes the mutant constitutively express the activity [23]. Importantly, binding to SHPS-1 enables SHP2 to de-phosphorylate Ras-GAP binding sites of PDGF-R at Tyr 771 [17] and EGF-R at Tyr 992 [16], thereby Ras signaling is promoted [16,17]. Since mitogenic $H_2O_2$ generates for 30–45 min [4], during which SHP2 acts on Ras-GAP binding site, the SHP2 appears tolerant to $H_2O_2$. Otherwise, mitogenic $H_2O_2$ quickly inactivates the SHP2. At present, it is entirely unknown how activated SHP2 can sustain its activity. If CAT binds SHP2, it could scavenge $H_2O_2$ and protect SHP2 from $H_2O_2$-dependent inactivation.

Recently, we have found that human CAT at 447pYVNV [24] closely resembles to 1374pYVNV of HGF-R [25,26], 1139pYVNQ of Erb2 [27–29] and 1213pYVNA of Flt-PTK [30,31], which commonly bind SH2-domain of GRB2, an adaptor protein that connects diverse signaling molecules [32]. Our data indicated that human CAT indeed binds GRB2 in vitro and co-immunoprecipitates with GRB2 upon integrin-stimulation in several cell lines [24]. In vitro GRB2 binding of wild type CAT, but not of Y447F, suggests that 447pYVNV is the binding site for GRB2-SH2 [24].

In this study, we further tested a hypothesis that CAT binds SHP2 via integrin-signaling to alter SHP2 sensitivity to $H_2O_2$. The results suggest that, like 988YIGV of PDGF-R, CAT-280YIQV binds SHP2–SH2 upon integrin-stimulation.

## 2. Materials and methods

### 2.1. Cells and reagents

A549 and tet-off HeLa cell line were obtained from ATCC and Clontech, respectively. pBI, pTK, pUC19M, and the transformer site-directed mutagenesis kit were from Clontech. Antibodies were from Calbiochem, Abcam, Santa Cruz Biotech, Qiagen, Promega or Amersham. Recombinant SHP2–SH2–GST fusion protein and

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*Abbreviations:* CAT, catalase; MAPK, mitogen-activated protein kinase; P-Tyr, phosphotyrosine; PTP, protein tyrosine phosphatase; RPTK, receptor protein tyrosine kinase; SH2, Src homology-2; PTB, phosphotyrosine binding domain; Gab-1, GRB2 associated binder-1; SHPS-1, SHP substrate-1; EGF-R, epidermal growth factor receptor; PDGF-R, platelet derived growth factor receptor; HGF-R, hepatocyte growth factor receptor; FBS, fetal bovine serum; DTT, dithiothreitol; PDI, protein disulfide isomerase; Ras-GAP, Ras-GTPase activating protein; BTAM, bisphosphoryl tyrosine-based activation motif; VEGF-R, vascular endothelial growth factor receptor

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GSH-agarose were from Santa Cruz Biotech. pCAT10 and pcDNA4/HisMax were from ATCC and Invitrogen, respectively, while pQE81L, pQETri System and Ni–NTA–agarose were from Qiagen. pCMV-SPORT6-SHP2 was from Invitrogen. Biotinylation kit was from Pierce. Other reagents were from Sigma or Calbiochem.

*2.2. Cell culture*

We used Ham's F-12 and DMEM supplemented with 10% FBS for A549 and HeLa cells, respectively. The media contained streptomycin (100 μg/ml) and penicillin (100 U/ml). In some cases, it contained G418 (400 μg/ml), hygromycin-B (400 μg/ml), and Tet (2 μg/ml).

*2.3. Immunoprecipitation*

Cells were lysed in a buffer consisting of 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM $Na_3VO_4$ 10 mM NaF, 0.5% Triton X-100, 0.5 mM AEBSF, 7.5 μM pepstatin A, 7 μM E-64,18 μM bestatin, 10.5 μM leupepsin, and 0.4 μM aprotinin. We omitted $Na_3VO_4$ for measurement of SHP2 phosphatase activities. We performed immunoprecipitation as previously reported [24].

*2.4. Site-directed mutagenesis*

To construct pUC19M/CAT-wild type, pcDNA4/HMCAT (85–1710) [26] was digested with *Kpn*I (nt: 1176) and *Xba*I (nt: 1235), and the resulting 1657 bp fragment was ligated with pUC19M linearized by *Kpn*I (nt: 408) and *Xba*I (nt: 423). To create Y280F mutant from pUC19/CAT-wild type, we used the Transformer Site-Directed Mutagenesis Kit together with three primers. The first primer corresponded to 5′-pC (nt: 873) C ATT GCC ACC GGT AAG TAC CCC TCC TGG ACT TTT TTC ATC CAG GTC (nt: 919)-3′, where the original sequence of TAC was altered to TTC with creation of AgeI site. The second corresponded to 5′-pG (nt: 1642) GCA AAT CTG AAGCTT CGG GGC CCT GCA CCT G (nt: 1673)-3′, where the stop codon was replaced by a HindIII site. The third corresponded to 5′-pG (nt: 175) AG TGC ACC ATG GGC GGT GTG AAA T (nt: 199)-3′. We performed two rounds of mutagenesis using *Escherichia coli* NM522 and DH5α.

*2.5. Preparation of recombinant wild type CAT and Y280F*

We constructed a bacterial expression vector for an 8×His-tagged CAT at the C-terminus in three steps. First, pUC19M-CAT or -Y280F was digested with *Bam*HI and *Eco*RI to produce a fragment of 188 bp (nts: 85–273). Second, the same clone was digested with *Eco*RI and *Hin*dIII to produce a fragment of 1379 bp (nts: 273–1652). Third, the both fragments were ligated to a pQETri System linearized with *Bam*HI (nt: 1290) and *Hin*dIII (nt: 1342). *E. coli* DH5α transformed with pQE81-CAT, pQETri-CAT or pQETri-CAT/Y280F was cultured with 1 mM IPTG overnight and lysed in a buffer consisting of lysozyme (1 mg/ml), 50 mM phosphate buffer (pH 8.0), NaCl (300 mM) and imidazole (10 mM), followed by sonication. CAT was purified using Ni–NTA–agarose as previously described [24].

*2.6. The effects of TNFα, integrin ligands, CAT levels, and 3-AT on CAT–SHP2 binding*

Establishment of the double stable Tet-off HeLa cells was reported earlier [24]. A549 or HeLa cells cultured overnight under FBS-deprivation, were incubated for 18–24 h with 10% FBS, laminin (5 μg/ml), hyaluronic acid (10 μg/ml), elastin (10 μg/ml), GRGDTP (2.93 μM), GRADSP (2.93 μM) or fibrinogen (4 ∼ 100 μg/ml). In some cases, A549 cells were cultured with 10% FBS containing TNFα. The effects of CAT levels and its activities on CAT–SHP2 binding were determined as previously reported [24].

*2.7. In vitro binding assay*

To determine the significance of CAT-280Y toward SHP2–SH2 binding, a recombinant SHP2–SH2–GST fusion protein (3 μg) was incubated for 2 h on ice with wild type CAT (3 μg), Y280F (3 μg), or human erythrocyte CAT (6 μg) in a lysis buffer (500 μl), followed by overnight incubation with GSH–agarose (10 μg). To evaluate competitive inhibition by wild type CAT, but not by Y280F, toward SHP2–SH2 binding, SHP2–SH2–GST (3 μg) was first incubated with wild type CAT (6.9 μg ) or Y280F (6.9 μg) together with GSH–agarose (15 μg) as described above. After rinsing with PBST, the agarose was then incubated for 4 h on ice with biotinylated wild type CAT (12 μg).

*2.8. Statistics*

To determine the effects of TNFα, CAT levels and its activities on SHP2-CAT binding, normalized band intensities were expressed as arbitrary numbers. SHP2 phosphatase activities were compared between control, DTT and $H_2O_2$ groups. The comparison between the groups was perfomed using ANOVA test followed by the Tukey–Kramer multiple comparison test. Statistical significance was measured at the level of $P = 0.05$.

## 3. Results

First, we perfomed co-immunoprecipitation experiments using A549 cells. We estimated that CAT binding of SHP2 might occur within a similar timeframe as for GRB2, in which the maximum binding occurred 18–24 h after FBS-stimulation [24]. To determine the time-course events, A549 cells cultured under FBS-deprivation overnight were stimulated with 10% FBS for various periods. The results indicated that, while CAT little associated with SHP2 under FBS-deprivation (Fig. 1A), the binding became discernible at 3 h and reached the maximum level at 24 h. Although the binding level depended on the FBS concentrations, calf serum was also effective to evoke the binding (data not shown). To determine whether integerin-ligands in FBS were responsible for the binding, we compared laminin, hyarulonic acid, elastin and fibrinogen with 10% FBS. The results indicated that laminin and fibrinogen, but not others, evoked binding (Fig. 1B and C) . In addition, TNFα negatively regulated the binding in a dose-dependent manner (Fig. 1D). Thus, CAT binding to SHP2 was a specific event of fibrinogen- or laminin-stimulation.

To investigate whether the binding occurs only with A549 cells, we conducted similar experiments using HeLa cells. As shown in Fig. 2A, HeLa cells cultured in 10% FBS displayed a discernible binding, whereas those under FBS-deprivation showed little association. In addition, herbimycin-A, a peptide containing integrin binding sequence, and its analogue, somewhat inhibited the binding. Likewise, whereas fibrinogen evoked binding even under FBS-deprivation, the both peptides and herbimycin-A again suppressed the binding. These results suggest that HeLa cells also evoke CAT binding to SHP2.

To determine the effects of CAT levels and its enzyme activities on the binding, we used a Tet-off HeLa cell line capable of inducing active CAT nearly 15-fold above basal levels upon Tet-deprivation from culture medium [24]. We determined the effects by comparing CAT levels co-immunoprecipitated with SHP2, rather than SHP2 levels co-immunoprecipitated with CAT, since the latter approach would underestimate the binding if induced CAT exists as a free form. The results indicated that the cells at a basal CAT level consistently formed the complex upon FBS stimulation (lane 1 vs. lane 3, Fig. 2B), and that the cells expressing a high CAT level substantially increased the binding even without FBS stimulation (lane 1 vs. lane 5, Fig. 2B). It suggests that the binding critically depends on CAT levels as observed for GRB2 binding [24]. Upon FBS stimulation, the binding further increased by nearly 2-fold compared to the level seen without FBS (lane 5 vs. lane 7, Fig. 2B). Furthermore, 3-AT significantly suppressed the binding (lane 5 vs. lane 6, and lane 7 vs. lane 8, Fig. 2B). These results suggest that active CAT levels critically determine the binding.

To examine the effects of CAT binding on SHP2 activity, we compared two SHP2 immunoprecipitates, that correspond to

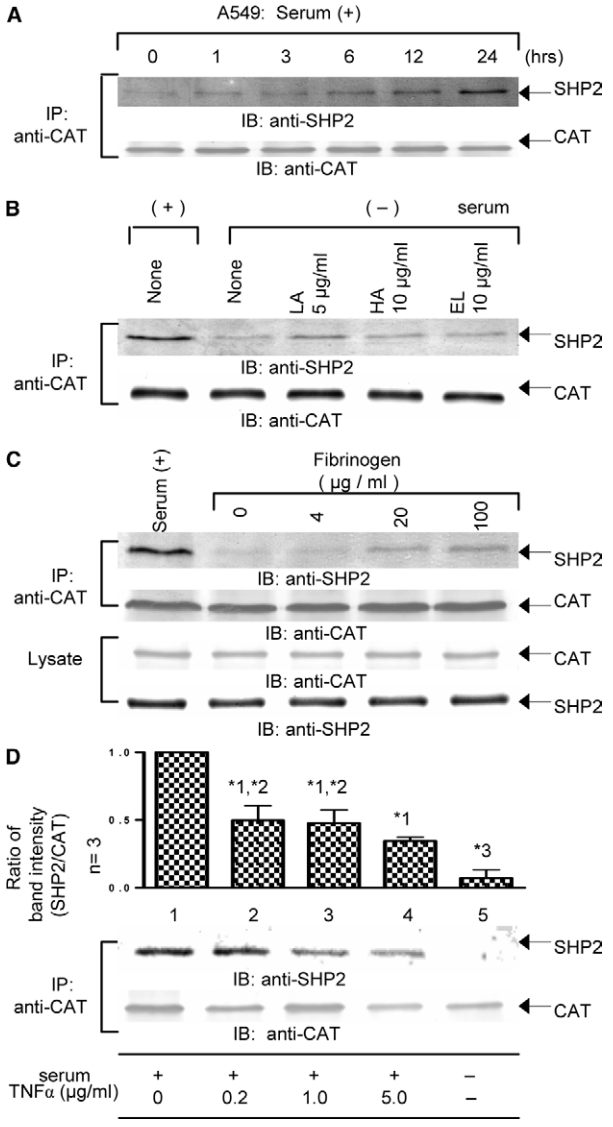


Fig. 1. (A) CAT binds SHP2 in A549 cells after FBS-stimulation. FBS-deprivation: overnight, 10% FBS: the period indicated. (B) CAT binds SHP2 upon stimulation with FBS or laminin (LA), but not with hyaluronic acid (HA) or elastin (EL). FBS-deprivation: overnight, 10% FBS or an agent indicated: 24 h. (C) CAT binds SHP2 in a fibrinogen-dose dependent manner. FBS-deprivation: overnight, 10% FBS or fibrinogen: 24 h. (D) TNFα negatively regulates CAT binding to SHP2. FBS-deprivation: overnight, 10% FBS with TNFα: 24 h.

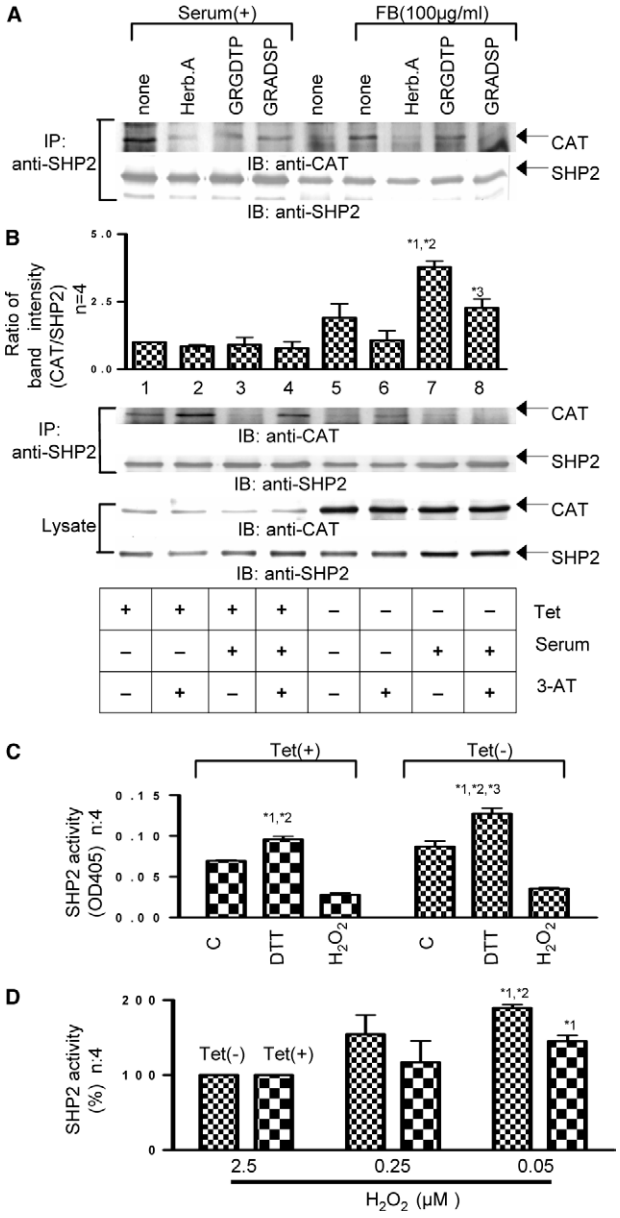


Fig. 2. (A) CAT binds SHP2 upon stimulation of HeLa cells with FBS or fibrinogen, while herbimycin-A and peptides comprising fibronectin functional sequence suppress the biding. FBS-deprivation: overnight. With 10% FBS, fibrinogen (FB), herbimycin-A (Herb.A, 5 μM), GRGDTP (2.93 μM) or GRADSP (2.93 μM): 24 h. (B) CAT binds SHP2 depending on CAT levels and activities. Tet-off HeLa cell with or without Tet (2 μg/ml): 8 d. FBS-deprivation: 24 h. In the medium containing 10% FBS, 10 mM 3-AT or both: 18 h. *1; $P < 0.001$ vs. lanes 1–6, *2; $P < 0.01$ vs. lane 8, *3; $P < 0.05$ vs. lanes 1–4. (C) SHP2 associated with high CAT levels expresses high phosphatase activities compared to those with low CAT levels. Culture condition: same as in (B). Incubation: 30 °C for 30 min. Phosphatase assay mixture: 30 mM p-nitrophenyl phosphate, 62mM HEPES, 6.25 mM EDTA, pH 7.0. DTT; 3 mM DTT, $H_2O_2$; 5 μM $H_2O_2$. *1; $P < 0.01$, vs. control, *2; $P < 0.001$, vs. $H_2O_2$, *3; $P < 0.01$, vs. tet(+) DTT. (D) SHP2 associated with high CAT levels show $H_2O_2$-resistant activities. *1; $P < 0.001$, vs. 2.5 μM $H_2O_2$, *2; $P < 0.01$, vs. tet(+) 0.05 μM $H_2O_2$.

the lane-3 and -7 in Fig. 2B. The two exhibited identical SHP2 levels yet difference in their binding levels as shown in Fig. 2B. The results indicated that the SHP2 with high levels of CAT displayed significantly high phosphatase activities compared to those with little CAT in the absence and presence of 3 mM DTT (Fig. 2C). Despite of these differences, all samples showed substantially low but nearly identical phosphatase activities in the presence of 5 μM $H_2O_2$ (Fig. 2C). A dose-response analysis indicated that SHP2 became inactivated as $H_2O_2$ concentration increased, and importantly, the SHP2 associated with high levels of CAT exhibited significantly high phosphatase activities compared to those with little CAT in the presence of 0.05 μM $H_2O_2$ (Fig. 2D). Thus, SHP2 is indeed redox-sensitive as previously reported [10–13] and CAT binding renders SHP2 resistant to $H_2O_2$.

Next, we examined whether CAT comprises a sequence identical to any established SHP2 binding motifs. We found that CAT 280YIQV resembled to 988YIGV of human PDGF-R, which binds SHP2–SH2 [17]. To determine the significance

of CAT 280YIQV for the binding, we compared wild type CAT with Y280F. Successfully constructed clones of pQE81L showed IPTG-dependent expression of CAT tagged with 6×His at N-termini, and reacted with anti-CAT IgG and Ni–NTA-HRP (Fig. 3A). In contrast, the clones of pQETri showed leaky expression of CAT tagged with 8×His at C-termini (Fig. 3A). To purify wild type CAT and Y280F using Ni–NTA-conjugated agarose, we tested two extraction conditions, one with 8M urea to denature the CAT, and, the other using lysozyme and sonication to preserve the native form. The resin retained only CAT tagged at C-termini but not those at N-termini under native form (Fig. 3B), suggesting that C-terminal 8×His tag, but not N-terminal 6×His, of native CAT is accessible to the resin. To determine the difference between wild type CAT and Y280F toward SHP2–SH2 binding, we performed an in vitro binding assay using a recombinant SHP2–SH2–GST fusion protein. The results indicated that wild type CAT and human erythrocyte CAT indeed bound SHP2–SH2–GST directly, while Y280F failed to do so in vitro (Fig. 3C). To ascertain the difference, we determined the competitive inhibition toward SHP2–SH2 binding. The results indicated that, whereas SHP2–SH2 consistently bound wild type CAT regardless of whether CAT was biotinylated or not, pre-incubation of SHP2–SH2 with non-biotinylated wild type CAT substantially blocked binding of biotinylated wild type CAT to the SHP2 (Fig. 3D). Importantly, pre-incubation with Y280F little affected the subsequent binding of biotinylated wild type CAT (Fig. 3D). These results demonstrate that CAT-280YIQV is a SHP2–SH2 binding site.

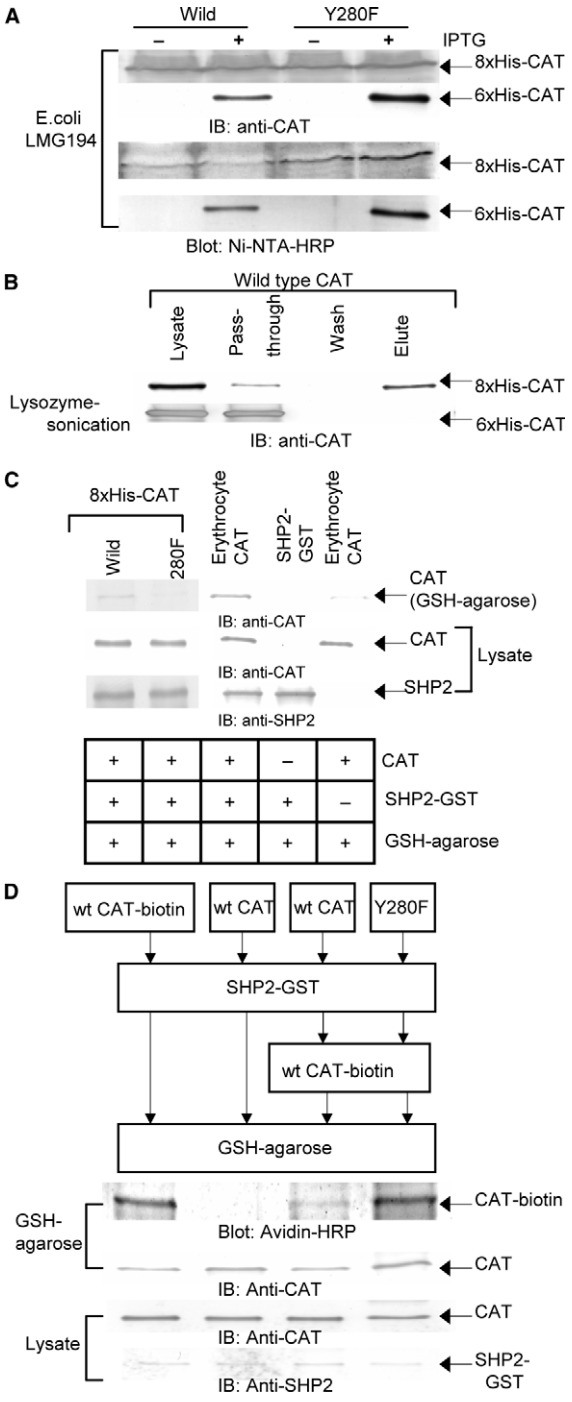


Fig. 3. (A) IPTG-dependent expression of wild and mutant CAT tagged with 6×His at N-termini or 8×His at C-termini. (B) Ni–NTA–agarose retains CAT tagged wife 8×His, but not those with 6×His. (C) Recombinant full-length wt CAT and human erythrocyte CAT, but not Y280F, bind SHP2–SH2–GST. (D) A pre-incubation of SHP2–SH2–GST with wt CAT, but not wife Y280F, inhibits a further binding of SHP2–SH2–GST to biotinylated wt CAT.

## 4. Discussion

SH2-binding [16,17,19–22] and a redox-status at the active center [14,15] regulate SHP2 activity. In fact, a mutant lacking SH2 domain constitutively expresses phosphatase activity, and binding of SH2 domains activates the enzyme. It suggests that N-terminus folding into the catalytic domain and its release control the activity. In addition, all known PTP including SHP2 commonly comprise a single sulfhydryl group at the active center [14,15] and become inactive upon its oxidation. The current data newly suggest CAT-binding based regulation of SHP2 activity.

The results raise several possibilities and questions. *First*, one might wonder why the event is so slow. Unlike other receptors, integrals play bi-directional regulatory roles known as outside-in and inside-out signaling through alteration of their conformation [34–37] via redox-exchanges. Indeed, integrins β1 and β3, for instance, contain 58 and 56 cysteins out of 798 and 788 amino acids, respectively, with C-x-x-C motif displaying the sulfhydryl-disulfide exchange activity [38,39] as does extracellular PDI [40,41]. In addition, DTT reduction changes conformation of thrombospondin [42,43], and, perhaps, of fibronectin, laminin and fibrinogen, too, and increases cellular access to RGD, a functional sequence to bind integrin [41]. Thus, integrins binding of their ligands and subsequent signaling critically rely on sulfhydryl and disulfide formation at appropriate positions. While oxidants and reducing agents favor for disulfide and sulfhydryl formation, respectively, $H_2O_2$ inhibits integrin binding of its ligands [44,45] perhaps due to random disulfide formation hampering redox-exchanges at correct positions. Thus, the CAT–SHP2 binding would be slow if redox agents were unbalanced or insufficient for the exchanges within and between integrins and their ligands. Facilitation of the binding by active CAT induction and its suppression by 3-AT (Fig. 2B), support this view.

*Second*, where does CAT–SHP2 binding fit and play its role in the integrin-signaling? As shown in Fig. 4, integrins evoke

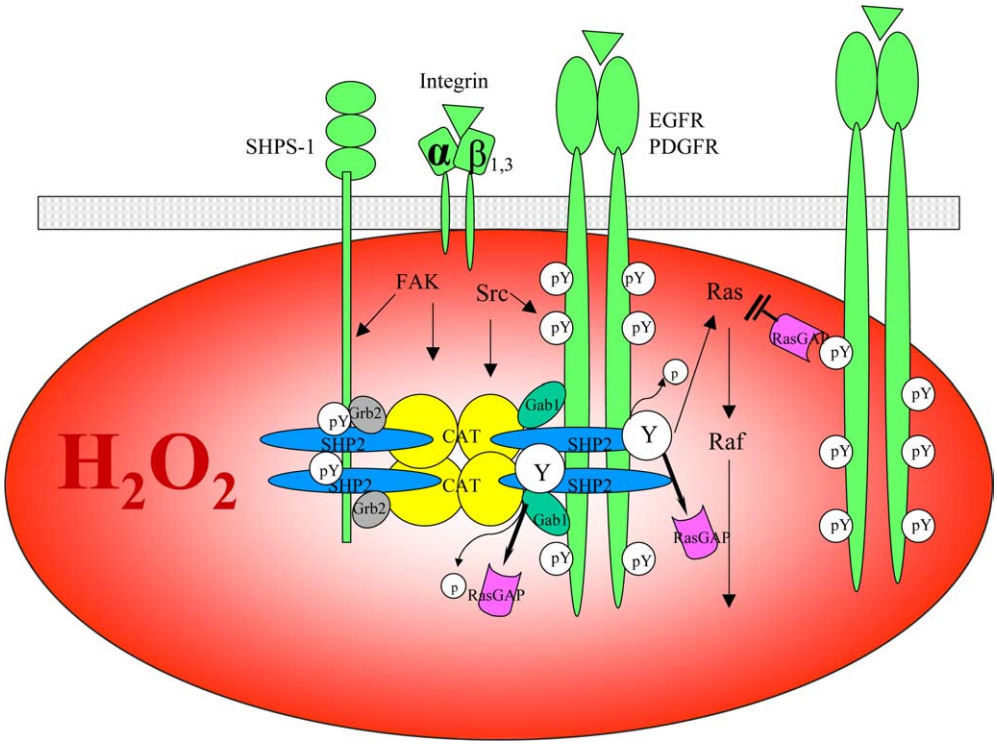


Fig. 4. Model of CAT interaction with SHP2, GRB2 and SHPS-1, and the effects on mitogenic signaling. Integrin activation evokes phosphorylation of SHPS-1 and CAT to form signaling aggregates that contain SHP2-CAT complex. Unlike other PTPs, the SHP2 complex resists to mitogenic $H_2O_2$ and dephosphorylates RasGAP binding sites of growth factor receptors, leading to Ras-signaling promotion.

phosphorylation of cytoplasmic domain of SHPS-1 to recruit SHP2 [20–22,46]. Upon EGF- or PDGF-stimulation, the SHP2 dephoshorylates P-Tyr at Ras-GAP binding site within EGF-R or PDGF-R, resulting in enhancement of Ras- and Erk-signaling [16,17]. At present, it is unclear how SHP2 recruited to SHPS-1 can work on the Ras-GAP binding sites upon EGF- or PDGF-stimulation that generates mitogenic $H_2O_2$, despite of its vulnerability to $H_2O_2$. One possibility is that, while BTAM of SHPS-1 or Gab1 binds SHP2 to activate, these molecules bind also SH3 of GRB2 [57], whose SH2 domain binds CAT 447pY [24]. Alternatively, N-SH2 of SHP2 might bind SHPS-1, PDGF-R, Gab1 or IRS-1, and SHP2-C-SH2 might bind active CAT. While CAT-280pYIQV is dissimilar to a consensus sequence, L/V-x-pY-x-D-L, of SHPS-1 (428pY and 469pY) [46], Gab1 (627pY) [19] and IRS-1 (1172pY) that bind SHP2-N-SH2, it resembles to 659pYVVV of Gab1 that binds SHP2-C-SH2. Thus, it is plausible that integrin-evoked SHPS-1 binding to SHP2 and GRB2 results in sequestering CAT, a tetramer of 60 kDa subunit [33] that can bind up to four SHP2 and GRB2, and some of the SHP2 may work on EGF-R or PDGF-R with a $H_2O_2$-resistance. Such a close proximity between integrins, SHPS-1 and a growth factor receptor, exists, because, upon ligation of integrin or R-PTKs, integrin-αVβ3 associates with PDGF-R, VEGF-R and insulin receptor [47–49] while integrin-α5β1 binds EGF-R [50–52]. Conversely, growth-factor stimulation preferentially activates integrin-associated R-PTKs [48] and R-PTKs activate membrane-proximal integrin-signaling [52]. Thus, CAT may enhance SHP2 activity and facilitate focal assemblage of integrin signaling molecules. A blockade of integrin-evoked CAT–SHP2 complex formation with TNFα (Fig. 1D), therefore, could be one of TNFα suppressive effects on tumor cell growth.

*Third*, it is likely that the binding occurs in tumor cells, but not in normal cells, despite that tumor cells display low CAT levels compared to non-tumor cells [53,54]. Whereas diverse origins of tumor cell lines commonly evoked CAT-GRB2 complex upon integrin-engagement, primary human bronchial epithelial cells as well as human diploid fibroblast cells displayed little binding [24]. In smooth muscle cells, overexpression of CAT suppresses mitogenic $H_2O_2$ and P-Tyr levels, leading little cell proliferation [4,8]. At present, it is not clear how the difference arises despite of common expression of integrins in all the cells. Interestingly, whereas C-terminal ANL should destine CAT to peroxisome, CAT induction in HeLa cells rather increased binding to GRB2 [24] and SHP2 (Fig. 2B). Several scenarios are possible. *First*, the peroxisome sequestration could be insufficient or impaired in tumor cells, displaying less peroxisome than non-tumor cells [55]. *Second*, the binding could be associated with undifferentiated or dedifferentiated cells such as peroxiome-naive cells at early development or tumor cells, for their growth. *Third*, integrin-dependent CAT expression [56] and its trafficking factors might have altered in tumor cells. It is also interesting to know how an increase of CAT–SHP2 complex upon CAT induction with oxidative or other insults, affects malignant cells attached on extracellular matrices such as fibrinogen, fibronectin and laminin.

In conclusion, the current study demonstrates that CAT 280YIQV binds SHP2–SH2 domain via integrin-signaling. The binding renders SHP2 resistant to $H_2O_2$. This is the first report indicating a direct binding of CAT to SHP2.

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