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Journal
Molecular Cancer Research, 11(9)

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Publication Date
2013-09-01

DOI
10.1158/1541-7786.MCR-12-0633

Peer reviewed

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Mol Cancer Res. Author manuscript; available in PMC 2014 September 01.

Published in final edited form as:
Mol Cancer Res. 2013 September ; 11(9): 995–1003. doi:10.1158/1541-7786.MCR-12-0633.

Characterization of B56γ tumor-associated mutations reveals mechanisms for inactivation of B56γ-PP2A

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Abstract

A subset of the hetero-trimeric PP2A serine/threonine phosphatases that contain B56, and in particular B56γ, can function as tumor suppressors. In response to DNA damage, the B56γ subunit complexes with the PP2A AC core (B56γ–PP2A) and binds p53. This event promotes PP2A-mediated dephosphorylation of p53 at Thr55, which induces expression of p21, and the subsequent inhibition of cell proliferation and transformation. In addition to dephosphorylation of p53, B56γ–PP2A also inhibits cell proliferation and transformation by a second, as yet unknown, p53-independent mechanism. Here, we characterized a panel of B56γ mutations found in human cancer samples and cancer cell lines and showed that the mutations lost B56γ tumor-suppressive activity by two distinct mechanisms; one is by disrupting interaction with the PP2A AC core and the other with B56γ–PP2A substrates (p53 and unknown proteins). For the first mechanism, due to the absence of the C catalytic subunit in the complex, the mutants would be unable to mediate dephosphorylation of any substrate and thus failed to promote both p53-dependent and p53–independent tumor-suppressive function of B56γ-PP2A. For the second mechanism, the mutants lacked specific substrate interactions and thus partially lost tumor-suppressive function, i.e. either p53-dependent or p53-independent contingent upon which substrate binding was affected. Overall the data provide new insight into the mechanisms for inactivation of tumor-suppressive function of B56γ and further indicate the importance of B56γ-PP2A in tumorigenesis.

Keywords

p53; PP2A; B56γ mutation; tumor suppression

Introduction

The protein phosphatase 2A (PP2A) is a family of serine/threonine phosphatases that is involved in a multitude of cell-signaling pathways. PP2A exists either in the cell as a heterodimer of scaffolding A subunit and catalytic C subunit (the AC core), or as a heterotrimeric complex where the AC core additionally associates with one of the variable B subunits. The B subunits have four gene families based on sequence homology: the B (B55 or PR55), B' (B56 or PR61), B" (PR48/59/72/130), and B"' (PR93/110). Each B subunit family contains two to five isoforms and many contain alternatively spliced variants. Binding of a specific B subunit determines diverse cellular localization and substrate

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specificities, allowing PP2A holoenzyme to have a diverse enzymatic activity in the cell (1, 2).

Recent evidence suggested that a subset of PP2A holoenzymes that contain B56 (B56-PP2A), in particular B56γ (PPP2R5C), functions as tumor suppressor (3, 4). Although the underlying mechanism is not fully understood, B56γ-PP2A is known to dephosphorylate and regulate specific substrates involved in cellular functions. For example, dephosphorylation of tumor suppressor p53 (TP53) at Thr55 activates p53, resulting in the induction of the CDK inhibitor p21 (CDKN1A), and inhibition of cell growth. However, in the absence of p53, B56γ-PP2A can still reduce cell growth, suggesting that it dephosphorylates other unknown substrates that also play roles in tumor suppression (4). Although the mechanism of the p53-independent function is unknown, additional proteins have been shown to interact with B56γ and potentially could be dephosphorylated by B56γ-PP2A. These proteins include the mitogen-activated kinase ERK (MAPK; 5), transcription co-factor p300 (EP300; 6), and centromeric cohesion recruited by Sgo1 (SGOL1; 7, 8). Overall these studies suggest that B56γ-PP2A acts as a tumor suppressor by dephosphorylating specific target substrates to regulate their effects on cellular functions. In support of this view, some viral oncoproteins function by displacing the B56 subunits from AC core (3, 9). In addition, mutations in PP2A Aα gene (PPP2R1A) and Aβ gene (PPP2R1B) identified in cancers are known to lose interaction with either the C subunit or the B56 subunits (10–13). Despite its importance in tumorigenesis, mechanisms for inactivation of B56γ-PP2A tumor-suppression by B56γ mutations are not well studied.

In this study, we characterized a panel of B56γ mutations previously identified in human tumor samples and cancer cell lines. Our results revealed three classes of mutations in the *B56γ* gene. The class I mutants, which includes A61V, A212T, S251R, E266R, P274T, H287Q and P289S, could bind to both the AC core and p53, and displayed a tumor suppressive activity similar to wild type (WT) B56γ, suggesting single mutations within this class have no effect on the B56γ tumor suppressor function. The class II mutants (C39R, E164K, Q256R and L257R) lost all B56γ-PP2A tumor suppressor activity, although still able to bind p53, they could not complex with the AC core. In contrast, the class III mutants (S220N, A383G and F395C) had a partial tumor suppressor activity compared to WT B56γ and could complex with the AC core. Two mutations, A383G and F395C, fail to bind to p53, thus explaining their loss of p53-dependent function (14). In contrast, S220N still bound and dephosphorylated p53 but reduced B56γ–PP2A tumor-suppressive activity, indicating that it disrupts p53-independent mechanism although the involved substrate is unknown. These results provide mechanistic insight into the inactivation of tumor-suppressive function of B56γ and further support the notion that multiple pathways are involved in B56γ-PP2A mediated tumor suppression.

Materials and Methods

Cell culture and plasmids

U2OS and HCT116 cells were cultured in McCoy's 5A medium supplemented with 10% fetal calf serum. The B56γ point mutants were generated using the QuikChange Site-Directed Mutagenesis Kit (Stratagene).

Western blot and immunoprecipitation

Whole-cell extract was prepared by lysing the cells in a buffer containing 50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% NP-40, 1 mM dithiothreitol, 2 μg/ml aprotinin and 2 μg/ml leupeptin. Cell lysates were subjected to SDS-PAGE, then analyzed by Western blot analysis using anti-p53 (DO1, Santa Cruz Biotechnology), anti-PP2A A subunit (Upstate),

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anti-PP2A C subunit (1D6, Upstate), anti-p21 (Santa Cruz Biotechnology), anti-PP2A B56γ (14), anti-HA (12CA5), anti-ERK (Santa Cruz Biotechnology), anti-SGOL1 (ABNOVA), anti-cyclin G (Santa Cruz Biotechnology), or anti-vinculin (VIN-11-5, Sigma) antibodies. For Thr55 dephosphorylation experiments, the cell lysate was immunoprecipitated with a phospho-specific antibody for phos-Thr55 (Ab202) and then immunoblotted with anti-p53 antibody (4). For interaction of endogenous proteins with transfected B56γ proteins, U2OS cells were transfected with various B56γ plasmids using FuGene (Roche) or BioT (Bioland Scientific) and lysed 28 h after transfection. Immunoprecipitation was performed using anti-HA monoclonal antibody. The amounts of co-precipitated proteins were determined by immunoblotting.

RT-PCR

Total RNA was extracted using Trizol reagent (Ambion), and RT-PCR was performed using SuperScript One-Step RT-PCR kit (Invitrogen) according to manufacturer's protocol. RT-PCR for p21 mRNA was performed with (F) 5'-CGACTGTGATGCGCTAATGG–3' and (R) 5'-GGCGTTTGGAGTGGTAGAAAT-3', and for GAPDH mRNA was performed with (F) 5'-AGGTGAAGGTCGGAGTCAAC-3' and (R) 5'-GACAAGCTTCCCGTTCTCAG-3'.

Identification of cancer-derived mutation

The NCBI AceView program (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html) provides a comprehensive sequence of the human transcriptome and genes of all quality-filtered human complementary DNA data from GenBank, RefSeq, dbEST, and Trace in a strictly complementary DNA-supported manner. Using this program, we looked for B56γ mutations in the annotated sequences of tumor samples and cancer cell lines.

Cell proliferation and anchorage-independent growth assays

To generate proliferation curves for HCT116 cells, cells were transfected with WT, mutant B56γ or a control cytomegalovirus (CMV) empty vector using BioT. Transfected cells were seeded in triplicate, and then counted at 120 h post seeding. The presence of overexpressed B56γ protein in the cell was verified by immunoblotting. For anchorage-independent growth assays, HCT116 cells were transfected with WT, mutant B56γ or a control empty vector seeded in triplicate in 0.35% Noble Agar (Fisher) and colony numbers were counted 4 weeks post seeding.

Results

Identification of potential tumor-derived mutations in B56γ gene

To better understand the role of B56γ in human cancers, we performed an AceView search for all of the known, tumor-associated mutations in the *B56*γ gene. AceView regularly downloads the whole set of cDNA sequences from the public databases, aligns them on the current genome available at NCBI, and clusters them into reference transcripts. Because all of the identified alternative transcripts were originally cloned from cancer samples or cancer cell lines, they represent potential tumor-inducing mutations. We identified twenty-four point mutations scattered throughout the *B56*γ coding region (Table 1; Figure 1), whereas a search of the NCBI single-nucleotide polymorphism (SNP) database did not yield any of these mutations. The fact that B56γ mutations are found in several different types of tumors (Table 1) suggests that B56γ may play a broad role in tumor suppression. The B56γ protein consists of eight pseudo Huntington-elongation-A subunit-TOR (HEAT) repeats. Interestingly, although the mutations are spread across the entire B56γ sequence, they cluster more frequently toward the center of the gene, notably on the HEAT-repeat 4, 5, 6

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and in a very small domain (aa 383–410) that contains the p53-binding domain (Figure 1), suggesting there are potential cancer mutation hot spots in the gene. The human B56γ transcript has at least three long splice variants known as γ1, γ2, and γ3 (15, 16) and most of the mutations we identified are common to all three variants. To begin investigating their function in tumor suppression, we used site directed mutagenesis to generate eleven of the new mutations (A61V, E164K, A212T, S220N, S251R, Q256R, L257R, E266R, P274T, H287Q, and P289S; Figure 1). All of these mutations, plus three previously reported C39R, A383G and F395C mutations, are shared by the three spliced isoforms and represent different clusters located in the *B56γ* gene (Figure 1).

Effect of identified mutations on the tumor-suppressive functions of B56γ

Because the mutations were identified in tumor samples, we assessed their effect on B56γ tumor suppressor activity. Previously, we showed that overexpression of WT B56γ inhibits cell proliferation and anchorage-independent cell growth in both p53-dependent and p53-independent manner (4). To evaluate the effect of the mutants, we first tested whether mutations affect the ability of B56γ to inhibit cell proliferation. Human colon cancer cells, HCT116 cells with either a $p53^{-/-}$ or $p53^{+/+}$ background, were transfected with WT B56γ or each of the mutants. As shown in Figure 2A, overexpression of WT B56γ in the presence of p53 (HCT116 $p53^{+/+}$ cells) led to approximately 45% decrease in cell number compared to the vector control after 120 h of cell growth. Similar level of decrease was also observed in a tetracycline-inducible B56γ overexpression U2OS cell line with $p53^{+/+}$ background (Supplemental Figure 1), suggesting it represented both p53-dependent and p53–independent inhibition. In contrast, in the $p53^{-/-}$ cells, overexpression of WT B56γ had a reduced effect on cell proliferation, with a 20% decrease in cell number, which represented the level of p53-independent inhibition.

In comparison, overexpression of B56γ mutants led to cell growth inhibition ranged from similar to WT, to partial reduction, to no inhibition at all. Based on their growth inhibition property, we classified all 14 mutants tested into one of three classes (Table 2). Class I, including A61V, A212T, S251R, E266R, P274T, H287Q and P289S, had little or no effect on B56γ-mediated growth inhibition in both HCT116 $p53^{+/+}$ cells and $p53^{-/-}$ cells, suggesting those individual single mutations have no effect on the B56γ tumor suppressor function. In contrast, Class II, including C39R, E164K, Q256R and L257R, were unable to inhibit cell growth in both $p53^{-/-}$ and $p53^{+/+}$ HCT116 cells, suggesting these mutants lost their ability to block cell proliferation regardless of p53 status. Class III, including S220N, A383G and F395C, only partially inhibited cell proliferation compared to wild type B56γ. As previously described, A383G and F395C show reduced inhibitory effect in $p53^{+/+}$ cells but not in $p53^{-/-}$ cells. This can be explained by their inability to bind and dephosphorylate p53 (14). Interestingly, S220N showed a partial inhibitory effect in $p53^{+/+}$ cells, but not in $p53^{-/-}$ cells, suggesting that this mutant specifically lost the p53-independent tumor suppressor activity of B56γ-PP2A.

To provide further evidence, we tested the effect of the mutations on anchorage-independent cell growth. Based on the result from cell proliferation assay (Figure 2A), we assayed A212T and P274T from Class I, E164K, Q256R, L257R from Class II, and S220N from Class III. HCT116 $p53^{+/+}$ cells and $p53^{-/-}$ cells were transfected with WT B56γ or the mutants, and seeded in soft agar. As shown in Figure 2B, overexpression of WT B56γ in $p53^{+/+}$ cells led to 65% reduction in the number of colonies compared to empty vector control, which represents both p53-dependent and p53-independent inhibition. In contrast, overexpression of WT B56γ in $p53^{-/-}$ cells only led to 18% decrease in colony numbers, which represents the p53-independent inhibition.

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When Class I A212T and P274T mutants were overexpressed, no significant changes in the number of colonies were observed compared to WT, suggesting those mutants have no effect on anchorage-independent growth suppression of B56γ. Class II E164K, Q256R and L257R mutants, however, completely abolished WT B56γ-mediated anchorage-independent growth suppression in both *p53*$^{-/-}$ and *p53*$^{+/+}$ cells, indicating that those mutations lost their ability to suppress anchorage-independent cell growth in both p53-dependent and – independent manner. Compared to WT, Class III S220N mutant partially lost its ability to inhibit anchorage-independent growth in *p53*$^{+/+}$ cells and completely lost its ability in *p53*$^{-/-}$ cells, suggesting that it specifically disrupts p53-independent function of B56γ. A383G and F395C from Class III were previously shown to specifically block p53-dependent function of B56γ (14). Taken together, our results demonstrate that Class II and III cancer-associated B56γ mutations disrupt, either completely or partially, tumor suppressive activity of B56γ.

B56γ mutations interfere with interaction with either the AC core or substrates

To understand the mechanisms for inactivation of B56γ tumor suppressive function, we next assayed the ability of the mutants to interact with the AC core and with p53. WT or mutant B56γ was expressed in U2OS cells, and their interaction with the AC core and p53 was assayed by immunoprecipitation. As shown in Figure 3A and summarized in Table 2, none of the eleven new mutations tested affect interaction of B56γ with p53. This is perhaps not surprising because most of the mutations are not located near the mapped p53-binding domain (aa 391–401). Furthermore, all Class I mutants (A61V, A212T, S251R, E266R, P274T, H287Q and P289S) showed little or no effect on the AC interaction (Figure 3), supporting the notion that these individual mutations do not affect the B56γ tumor suppressor function (Figure 2 and Table 2). To further prove this, we examined the effect of these mutations on p53 Thr55 dephosphorylation and function. Results of four representatives (A61V, A212T, E266R and P274T) are shown in Figure 3B. Overexpression of these mutants led to efficient dephosphorylation of p53 at Thr55 and activation of the p53 transcription target p21 at levels similar to WT B56γ3 (summarized in Table 2). Because p53 Thr55 is the only known residue that is directly dephosphorylated by B56γ-PP2A, we were unable to assess the effect of the mutations on other potential dephosphorylation by B56γ-PP2A. However, given their ability to fully support p53-independent tumor suppressive function (Figure 2), it is likely that Class I mutants fully support B56γ-PP2A dephosphorylation.

In contrast, Class II mutants (E164K, Q256R and L257R) remained bound to p53, but lost their ability to interact with the AC core (Figure 3A). In addition, C39R was also unable to bind to AC core (17). We note that all residues in this class, C39, E164, Q256, and L257, are not making any direct contact to A or C subunits according to B56γ–PP2A crystal structure (18, 19). However, they are located in close proximity from the interaction interface (Figure 3D). E164 residue is located within intra-loop of HEAT-repeat 3 and its negatively charged side chain is important to form hydrogen bond to E118 and R167. Mutation of E to K in this position would abolish these hydrogen bonds and thus destabilize intra-loop of HEAT-repeat 2 that mediates interaction with A and C subunits. Q256 and L257 residues are located within the second helix of HEAT-repeat 5. The polar side chain of Q256 points toward a helix of HEAT-repeat 4 and interacts with E216. In Q256R mutation, arginine has a positively charged side chain that is larger than glutamine and additionally contacts E213. Alternatively, the hydrophobic side chain of L257 points toward the first helix of HEAT-repeat 5. In L257R mutation, a larger side chain of arginine would protrude into the adjacent helix and a positive charge of arginine would induce further alterations in the environment. Both cases would result in displacement of helices, leading to rearrange the location of intra-loops that mediate the interaction between B56γ and AC core. Interestingly, all four residues

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are conserved among the B56 family isoforms, indicating the importance of these residues for maintaining the interaction between B56 and AC core.

Because the C subunit is required for PP2A catalytic activity, our data explain why the Class II mutants completely abolished all tumor-suppressive function of B56γ-PP2A (Figure 2 and Table 2). To support this view, we assayed their effect on p53 Thr55 dephosphorylation and induction of p21 (Figure 3B, 3C and Table 2). The assays showed that, unlike WT B56γ, overexpression of Class II mutants fail to induce dephosphorylation of p53 at Thr55 and activation of p53 transcription target p21. These results demonstrate that the interaction of B56γ with the AC core is required for B56γ-PP2A to dephosphorylate and activate p53. Consequently, Class II mutants have lost their tumor suppressive function through disruption of the AC core interaction and loss of catalytic activity.

Previous study has shown that two mutants in Class III, A383G and F395C, remain bound to AC core, but have lost their ability to bind and dephosphorylate p53, leading to disruption of the p53-dependent tumor suppressor activity of B56γ-PP2A (14). Interestingly, unlike A383G and F395C, S220N still interacted with p53 at a level comparable to WT B56γ. Importantly, the protein also promoted dephosphorylation of p53 at Thr55 and induced p21 expression to levels similar to WT B56γ (Figure 3B, 3C, and Table 2). These results suggest that p53 is unlikely responsible for partial loss of tumor suppressor activity of the S220N protein (Figure 2). Together, our results show that Class III mutations inactivate the tumor suppressor activity of B56γ-PP2A by preventing B56γ from binding to its substrates, thereby indicating the importance of B56γ in recruiting the AC core to substrate, so that B56γ-PP2A can function correctly.

S220N binds to B56γ interacting proteins

Because S220N specifically abolished the p53-independent tumor suppressor activity of B56γ-PP2A, we hypothesize that this may be due to its lack of interaction with another unknown substrate. Interestingly, S220 is located on a large concave surface of B56γ that is unoccupied by A and C subunits and leans toward the catalytic pocket of the C subunit (Figure 4A). It has been previously suggested that this open area may be important for recruiting substrates (18). To identify potential substrate that may bind to wild type B56γ but not S220N, we assayed the ability of the S220N mutant protein to interact with several known B56γ interacting proteins including ERK, Cyclin G2 (CCNG2), Shugoshin 1 and p300 (Figure 4B and data not shown). The assay showed that the interaction of S220N with all of these proteins was similarly to WT B56γ (Figure 4B), suggesting that interactions with these proteins are unlikely to be responsible for loss of p53-independent tumor suppressor activity of S220N. Nevertheless, our results indicate that Class III mutations specifically abolished individual substrate interaction, thus partially disrupting B56γ tumor suppressor activity. Furthermore, our results also indicate that B56γ contains multiple substrate-binding domains, implying the role of a specific B subunit in multiple pathways. Further study will provide insight into these pathways and their role in PP2A-dependent tumor suppression

Discussion

In this study, we characterized a panel of B56γ mutations that were previously identified in human cancers and defined the molecular mechanisms behind loss of B56γ-PP2A tumor suppression. Since B56γ has been suggested to be a tumor suppressor gene and function as a B56γ-PP2A complex to dephosphorylate proteins involved in cancer, mutants that lost their interaction with either AC core or substrates could potentially affect its tumor suppressive function. Indeed, we have shown, the mutations could be categorized into three groups: “no effect” (Class I); “loss of AC core interaction” (Class II); and “loss of substrate interaction” (Class III) (Figure 5). The “loss of AC core interaction” group (C39R, E164K, Q256R and

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L257R) failed to bind to AC core and thus disrupted the B56γ-PP2A complex in the cells. This leads to the loss of all B56γ-PP2A tumor suppressor-related functions. As a result, overexpression of these mutants failed to promote p53-dependent and p53-independent tumor-suppression. Interestingly, the "loss of AC core interaction" mechanism has been observed with several previously described A subunit tumor-associated mutations and SV40 ST antigen, emphasizing the importance of the B56γ-PP2A complex in cancer suppression. In contrast, the "loss of substrate interaction" group (S220N, A383G and F395C) remains bound to the AC core, but failed to bind B56γ-PP2A substrates. The A383G and F395C mutations are located at the p53-binding domain, and thus caused the loss of p53 interaction, which specifically abolished p53-dependent B56γ tumor suppressor function. In contrast, the S220N mutation is located at another potential substrate binding domain. Although we have not identified the substrate(s) involved in this loss of B56γ–PP2A function, we hypothesize that an unknown protein(s) plays a role in p53-independent tumor suppression. Taken together, our data defined detailed mechanisms for the inactivation of B56γ-PP2A in cancer. Given the fact that increased B56γ protein level is required for its function (4), those inactivation mechanisms may also apply for mutations that are heterozygous.

Although the B56γ mutations identified so far spread across the entire B56γ sequence, they appear more frequently in two regions: the center of the B56γ gene (HEAT-repeat 4, 5, and 6) and a small region toward the C-terminus (Figure 1). These clusters suggest possible existence of hot spots that are more susceptible to tumor-associated mutations. The existence of hot spots for somatic mutations often indicates that the region is essential for the function of protein. Indeed, the small region at the C-terminus (aa 383–401) contains a domain crucial for p53 binding (14), arguing that p53 is an important substrate for B56γ-PP2A function. Interestingly, although eleven mutations are located on HEAT-repeat 4, 5, and 6 alone, the majority of them did not affect the B56γ ability to inhibit cell growth. Aside from sequencing errors, it is possible that more than one point mutation is needed to change the protein function supported by those repeats. In fact, multiple interacting interfaces have been suggested for the complex formed between B56γ and the A and C subunits (18, 19). Further study of this region may lead to a better understanding of those interfaces and their roles in PP2A function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Margaret Morgan for careful editing of this manuscript.

Grant Support

This work was supported by the NIH grant R01CA075180 (to X. Liu) and by the American Heart Association Postdoctoral Fellowship POST3530033 (to Y. Wu)

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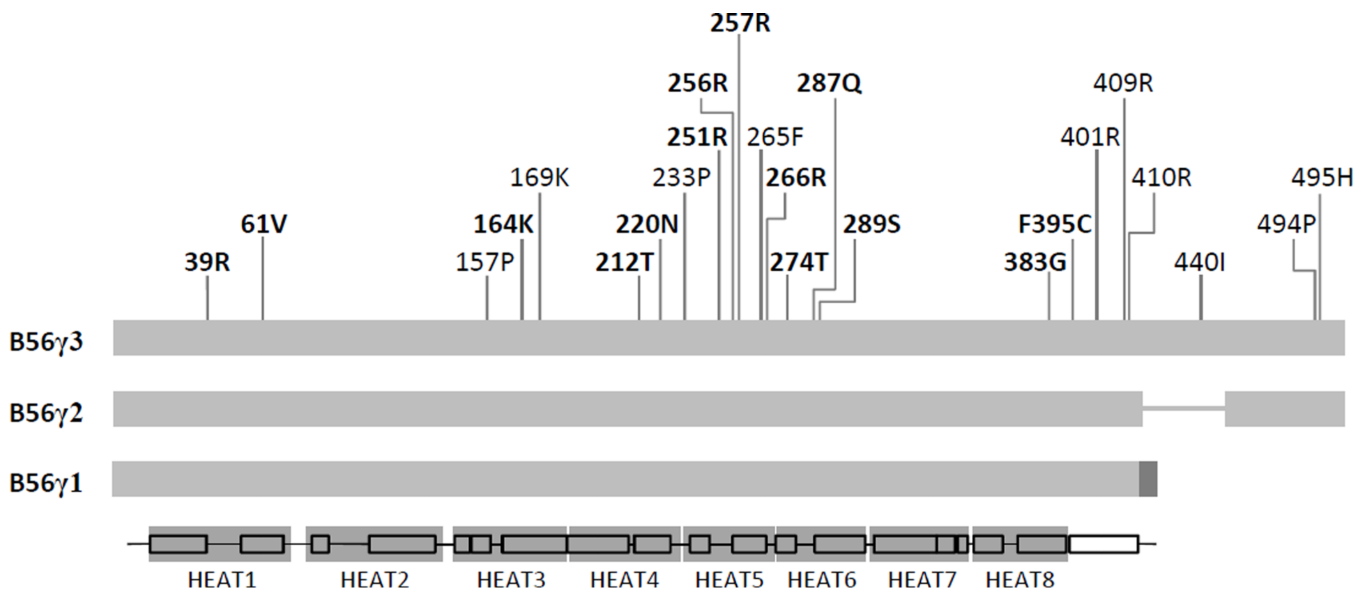

Figure 1. Distribution of B56γ mutations identified by AceView

Distribution of twenty-four mutations on three splice-variants, known as γ1, γ2, and γ3, of B56γ. Structure of B56γ is shown below. Each shaded rectangle represents a HEAT-repeat and each box represents a α-helix. Mutants in bold were chosen for characterization.

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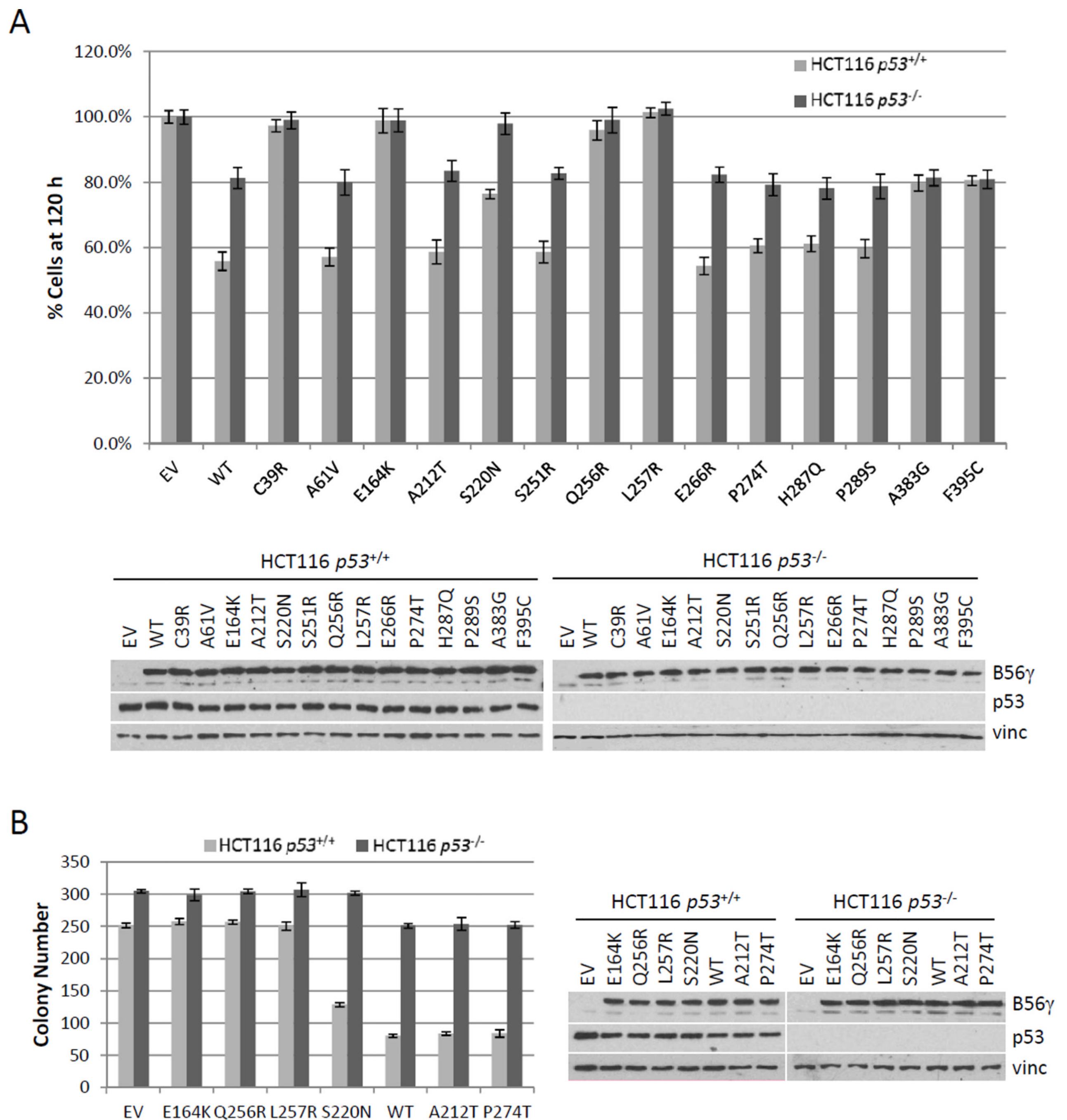

Figure 2. Effect of B56γ mutants on tumor suppressor function

HCT116 human colon cancer cells with *p53*$^{+/+}$ or *p53*$^{-/-}$ background were transfected with HA-tagged WT or B56γ mutants. For the control (EV), cells were transfected with an empty cytomegalovirus (CMV) vector. (A) Representatives of cell proliferation assay where transfected cells were seeded and harvested and counted after 120 h of growth. Numbers of cells were normalized against the representative empty vector controls and plotted in a bar graph. Error bars show average ± s.d. from triplicate plates in one representative experiment. Cells harvested were lysed and protein expression for endogenous B56γ (lower), HA-B56γ (higher), p53 and vinculin (vinc) were analyzed by western blot. (B) Representatives of anchorage-independent growth assay where transfected cells were seeded in soft agar and

number of colonies were counted. Error bars show average ± s.d. from triplicate plates in one representative experiment. Cells at initial seeding were lysed and analyzed for B56γ protein expression.

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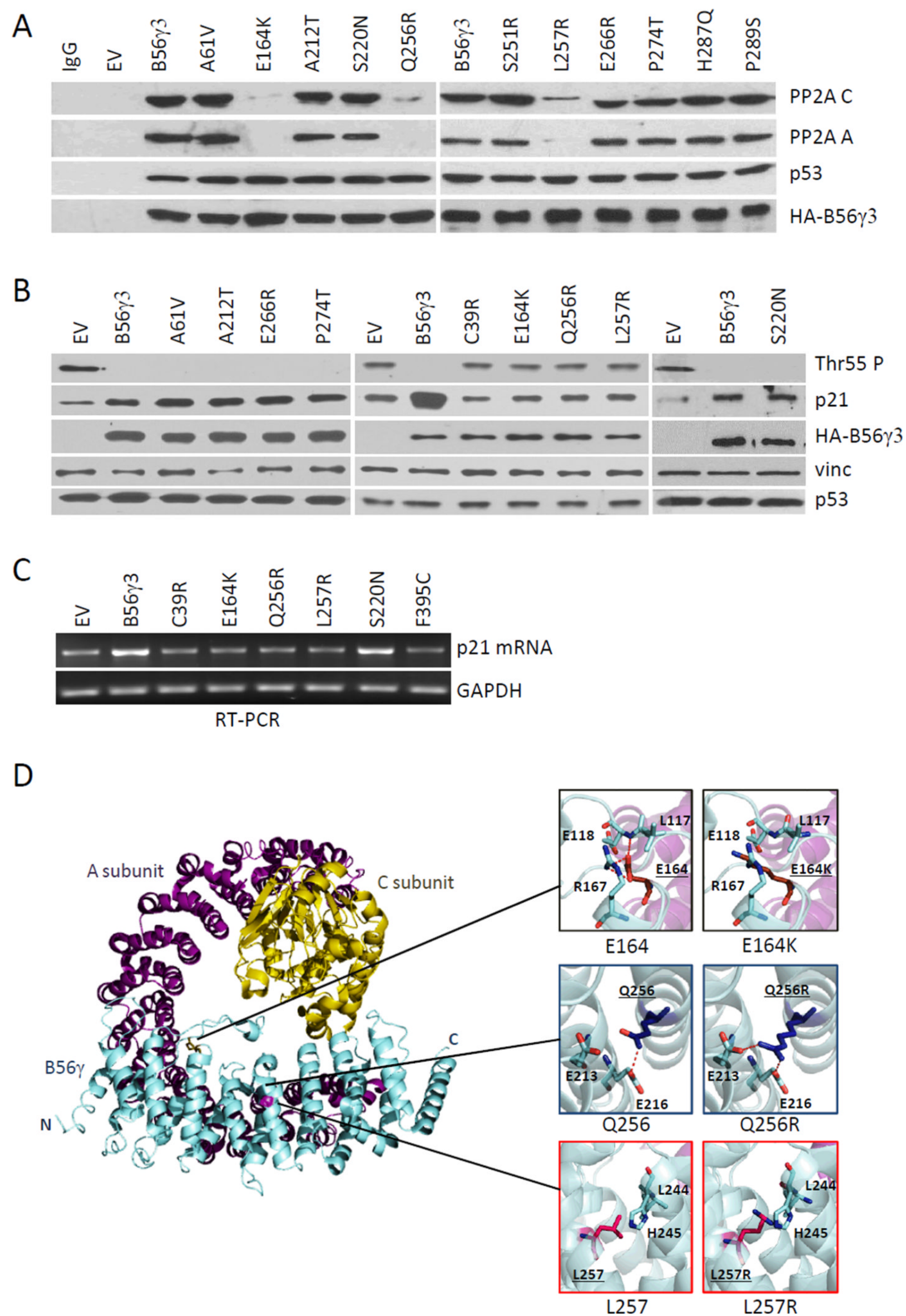

Figure 3. Interaction of B56γ mutants with PP2A A and C and p53

U2OS cells were transfected with empty vector control (EV), HA-tagged WT or mutant B56γ. (A) WT and mutant B56γ were immunoprecipitated and interacting proteins were analyzed by western blot using antibodies listed. (B) p53 Thr55 dephosphorylation, p21 protein levels, p53, and vinculin (vinc) were analyzed by western blot. p53 Thr55 phosphorylation levels were analyzed by phospho-specific antibody for Thr55 in presence of MG132. The p21 protein levels were tested in the absence of MG132. (C) The p21 mRNA levels were analyzed by RT-PCR. (D) Class II mutations are shown on the crystal structure of B56γ-PP2A holoenzyme (adapted from Protein Data Bank, accession code 2NYM),

prepared by PyMOL. The PP2A holoenzyme is displayed with A, C, and B56γ. Mutation residues are indicated for E164, Q256, and L257. Dashed lines indicate hydrogen bonding.

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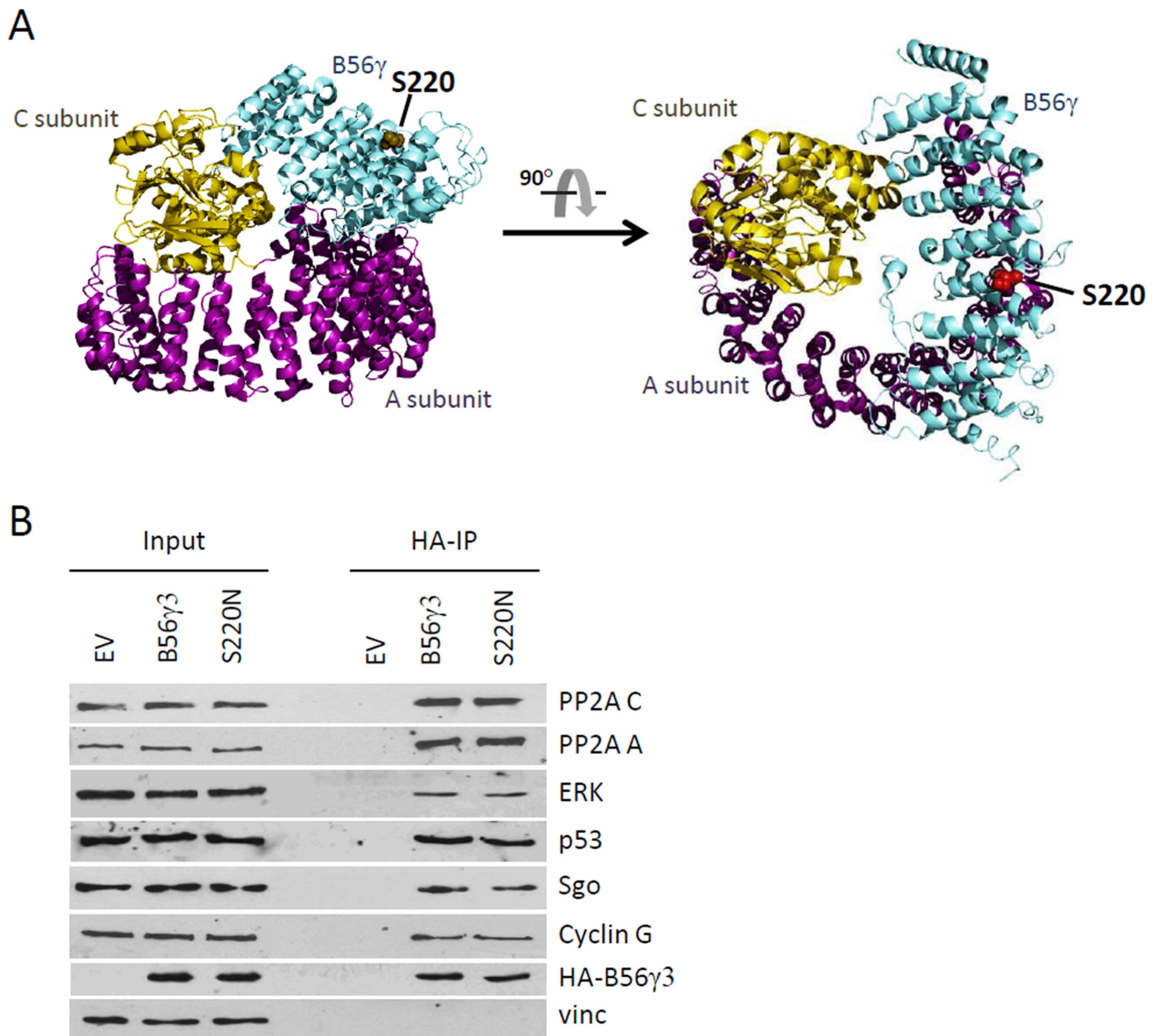

Figure 4. S220N interacts with AC core and p53

(A) S220 is indicated on the crystal structure of B56γ-PP2A holoenzyme. (B) Lysates of U2OS cells that were transfected with empty vector control (EV), HA-tagged WT or S220N B56γ were immunoprecipitated with anti-HA antibody, then analyzed by western blot against PP2A A and C, ERK, p53, Sgo, Cyclin G, HA, and vinculin (vinc).

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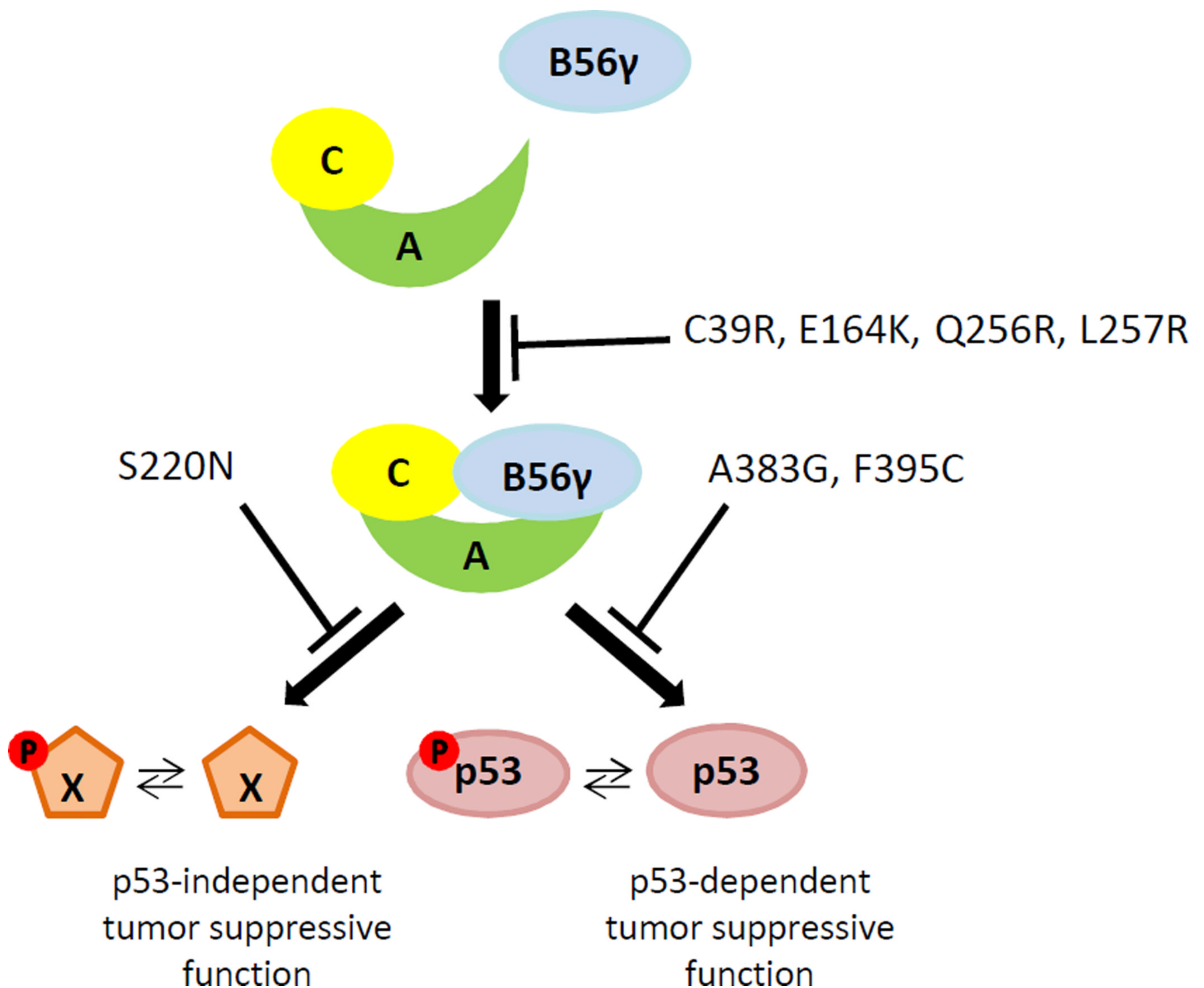

Figure 5. Inactivation of B56γ-PP2A tumor-suppressive function by B56γ mutations

The Class II mutations C39R, E164K, Q256R and L257R failed to bind to AC core and thus disrupted all B56γ-PP2A tumor suppressor-related functions. The Class III mutations (S220N, A383G and F395C) failed to bind B56γ-PP2A substrates and thus partially lost tumor-suppressive function of B56γ-PP2A.

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Table 1

Amino acid (protein)	Isoforms	Origin	HEAT-repeat	Reference
C39R	γ1, 2, 3	Pooled glandular	1	(17)
A61V	γ1, 2, 3	Pooled glandular	1	This study
L157P	γ1, 2, 3	Pooled glandular	3	
E164K	γ1, 2, 3	Pooled glandular	3	This study
R169K	γ1, 2, 3	Pooled glandular	3	
A212T	γ1, 2, 3	Melanotic melanoma skin	4	This study
S220N	γ1, 2, 3	Leiomyosarcoma uterus	4	This study
H233P	γ1, 2, 3	Embryonal carcinoma	5	
S251R	γ1, 2, 3	Embryonal carcinoma	5	This study
Q256R	γ1, 2, 3	Melanotic melanoma skin	5	This study
L257R	γ1, 2, 3	Melanotic melanoma skin	5	This study
L265F	γ1, 2, 3	Embryonal carcinoma	5	
E266R	γ1, 2, 3	Embryonal carcinoma	5	This study
P274T	γ1, 2, 3	Large cell carcinoma lung	6	This study
H287Q	γ1, 2, 3	Pooled glandular	6	This study
P289S	γ1, 2, 3	Pooled glandular	6	This study
A383G	γ1, 2, 3	Ilea mucosa	8	(14)
F395C	γ1, 2, 3	Lung carcinoma	9	(14)
Q401R	γ1, 2, 3	Pooled germ cell tumor	9	
E409R	γ1, 2, 3	Pooled germ cell tumor	9	
K410R	γ1, 2, 3	Pooled germ cell tumor	9	
S440I	γ1, 2, 3	Lung carcinoma		
H494P	γ2, 3	Embryonal carcinoma		
T495H	γ2, 3	Embryonal carcinoma		

Different tumor-derived B56γ mutations shown with the mutated residue (column 1), applicable B56γ isoforms (column 2), tumor origin of the mutation (column 3), location on the B56γ protein (column 5), and reference (column 6).

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Table 2

B56γ3	Interaction		p53 Thr55 dephosphorylation	p21 Induction	Cell Growth Inhibition	
	PP2A AC core	p53			p53 dependent	p53 independent
WT	Y	Y	Y	Y	Y	Y
C39R	N	Y	N	N	N	N
A61V	Y	Y	Y	Y	Y	Y
E164K	N	Y	N	N	N	N
A212T	Y	Y	Y	Y	Y	Y
S220N	Y	Y	Y	Y	Y	N
S251R	Y	Y	Y	Y	Y	Y
Q256R	N	Y	N	N	N	N
L257R	N	Y	N	N	N	N
E266R	Y	Y	Y	Y	Y	Y
P274T	Y	Y	Y	Y	Y	Y
H287Q	Y	Y	Y	Y	Y	Y
P289S	Y	Y	Y	Y	Y	Y
A383G	Y	N	N	N	N	Y
F395C	Y	N	N	N	N	Y

Summary of fourteen tumor-derived B56γ mutations' tumor-suppressive functional assays shown interaction of B56γ with PP2A AC core or p53 (column 2 or 3), dephosphorylation of p53 at Thr55 (column 4), induction of p21 (column 5), and effects on cell growth in p53-dependent or p53-independent manner (column 6 or 7), Y: Inhibition of cell growth; N: No inhibition on cell growth.