Subcellular localization of mutated β-catenins with different incidences of cis-peptide bonds at the Xaa246-P247 site in HepG2 cells

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ABSTRACT: Mutations may ultimately change the local conformation of proteins; however, little attention has been paid to alterations in protein function caused by the incidence of cis-peptide bonds (ICPB) in mammalian cells. In this study, a statistical approach, coimmunoprecipitation, and immunofluorescence staining have been used to confirm that S246→Y and S246→W missense mutations, which help increase the ICPB in Xaa246-P247 (Xaa is any amino acid) in human β-catenin, can reduce the interactions between β-catenin and adenomatous polyposis coli (APC) and between β-catenin and Ca2+-dependent cell adhesion molecule family in epithelial tissue (E-cadherin), eventually leading to increased nuclear migration of β-catenin in the HepG2 cell line (an immortalized cell line consisting of human liver carcinoma cells). Conversely, S246→L and S246→M missense mutations, which reduce the ICPB in Xaa246-P247 in human β-catenin, can enhance interactions between β-catenin and APC and between β-catenin and E-cadherin, leading to decreased nuclear migration of β-catenin. These results not only indicate that a change in the ICPB may be an important cause of functional protein changes but also provide a new basis for the study of genetic disease prediction, gene diagnosis, individualized treatment, and protein modification at the gene level for clinicians and other professionals.—Yu, S., Zhang, Y., Wu, Y., Yang, H., Chen, Y., Yang, Y., Zhang, Z. Subcellular localization of mutated β-catenins with different incidences of cis-peptide bonds at the Xaa246-P247 site in HepG2 cells. FASEB J. 33, 6574–6583 (2019). www.fasebj.org

KEY WORDS: missense mutations · ICPB · interaction · APC · E-cadherin

Peptide bonds are partial double bonds; thus, the dihedral angle ω with a peptide bond as the rotational axis is limited to only −180° or 0° (1). In 1970, the International Union of Pure and Applied Chemistry defined a dihedral angle ω equal to 180° as a trans-peptide bond and ω equal to 0° as a cis-peptide bond (2). More than 99% of the C=N bonds in amino acid polypeptide chains in human proteins are trans-peptide bonds, and <1% are cis-peptide bonds (3). Because the cis-trans isomerism of proteins is a slow conformational change, it has important functions in the regulation of changes in protein structure and function (4). Many regulatory processes in biologic activities, such as protein stability (1, 5), protein interactions (6, 7), membrane protein binding (8, 9), ion channels (10), cell signaling pathways (11, 12), gene expression (13), cancer development (14), amyloid peptide formation (15, 16), and Alzheimer’s disease (17, 18), are closely associated with cis-trans isomerism and the distribution of protein cis-peptide bonds. It has been reported that cis-trans isomerism results in changes in the spatial distance and location of functional groups on both sides of the peptide chain (5). Therefore, it is necessary to study the relationship between the distribution of protein cis-peptide bonds and protein function.

Genetic missense mutations can lead to amino acid substitution in the polypeptide chain, yet most substitutions will not change the protein structure or function; only a few mutations may change the protein structure or function. It is still not known whether alterations in protein

ABBREVIATIONS: APC, adenomatous polyposis coli; co-IP, coimmunoprecipitation; E-cadherin, Ca2+-dependent cell adhesion molecule family in epithelial tissue; ICPB, incidence of cis-peptide bonds; PDB, Protein Data Bank; Xaa, any amino acid; XnP, any amino acid other than Pro

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structure and function are caused by missense mutations related to the incidence of cis-peptide bonds (ICPB). Many studies have shown that the structure and function of proteins can be changed because of Xaa→P or P→Xaa point mutations (where Xaa is any amino acid) in polypeptide chains (1, 5). For regulated proteins, such as transcription factors and enzymes, only a few protein molecules with conformational changes may lead to significant changes in cell metabolism. These studies suggest that alterations in protein ICPB may be an important mechanism for changes in protein structure and function due to gene missense mutations. Therefore, a study of the distribution characteristics of cis-peptide bonds and the ICPB between 2 adjacent amino acids in a protein polypeptide chain consisting of 20 human amino acids is helpful for predicting changing trends in protein structure and function when the residues in the polypeptide chain are replaced with other residues because of missense mutations.

β-Catenin is an important core protein in the wingless/integrated (Wnt)–β-catenin signaling pathway, and it has also been reported that a “S246-P247” site in the middle of the β-catenin protein in cells can be recognized by peptidylprolyl cis-trans isomerase I to catalyze cis-trans isomerization. After phosphorylation modification, this site can be recognized by peptidylprolyl cis-trans isomerase I, which catalyzes an increase in the number of cis–β-catenin (S246-P247) molecules in the cell nucleus (20). Because β-catenin with a cis–S246-P247 peptide bond cannot interact with adenomatous polyposis coli (APC), it cannot be transported to the cytoplasm for degradation, eventually causing the accumulation of cis–β-catenin (S246-P247) molecules in the cell nucleus, which enhances the transcription of downstream genes. In addition, conformational changes in β-catenin S246-P247 may also lead to changes in the interactions between β-catenin and Ca2+-dependent cell adhesion molecule family in epithelial tissue (E-cadherin), leading to the release of β-catenin from the β-catenin–E-cadherin complex inside the cell membrane into the cytoplasm and ultimately an increase in the number of β-catenin molecules moving in the nucleus (21–24).

Above all, in this study, we used human β-catenin as a model protein to investigate the functional changes caused by alterations in the ICPB at Xaa246-P247 in mutated β-catenins. It is expected that our results will provide new insight for elucidating the mechanisms underlying protein functional changes induced by missense mutations at the DNA level.

**MATERIALS AND METHODS**

**Definition of cis-peptide bonds and the establishment of a nonredundant data set**

According to the systematic naming principle of organic chemicals proposed by the International Union of Pure and Applied Chemistry, if 2 identical atoms or groups of atoms are arranged on the same side of the double bond, they are called cis isomers; if 2 identical atoms or groups of atoms are arranged on either side of a double bond, they are called trans-isomers. Thus, in this study, a cis-peptide bond was defined as having an angle of $0^\circ < \omega < 90^\circ$, and the trans-peptide bond was defined as having an angle of $90^\circ < \omega < 180^\circ$. From the protein structure data released by the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) on October 12, 2017, the human protein data set was obtained using X-ray experimental methods with a resolution <2.0 Å and an identity <30%. After redundant sequences were removed, our data set contained 1614 proteins.

**Statistics for cis- and trans-peptide bonds and the ICPB of Xaa-P**

We designed a C program and statistically analyzed the distribution of cis- and trans-peptide bonds in our data set and Xaa-P in cis-peptide bonds and then calculated the abundance of 20 Xaa-P peptide bonds and their cis-peptide bonds in our data set. Finally, we calculated the ICPB of Xaa-P cis-peptide bonds.

**Analysis of the amino acid residues surrounding each cis-peptide bond**

The $z$-score method was performed to analyze the abundance of amino acid residues surrounding each cis-peptide bond in our data set and their effect on the ICPB. Here, we calculated the $z$ score for every amino acid residue in segments that included 5 residues before and after each cis-peptide bond in our data set.

The formula was as follows:

$$Z_i(a) = \frac{Xaa_i(a) - \mu}{\sigma}$$

where $Z_i(a)$ is the $z$ score of the $i$th amino acid in 1 segment, Xaa is any amino acid residue of 1 segment, $Xaa_i(a)$ is the frequency of Xaa at the $i$th position, $\mu$ is the mean value of all the residue frequencies of the segments, and $\sigma$ is the sd. The specific method is shown in refs. 25 and 26.

**Statistical analysis of non-Pro cis-peptide bonds (Xaa-XnP)**

After the Xaa-P peptide bonds in all of the Xaa-Xaa cis-peptide bonds were removed; the type, number, and distribution rate of different Xaa-XnP (XnP is any amino acid other than P) cis-peptide bonds were analyzed; and then, the distribution pattern of these cis-peptide bonds was obtained.

**Plasmid construction and lentiviral packaging**

To further investigate the association between the alteration of the ICPB and functional changes in the model proteins in eukaryotic cells, the β-catenin gene fragment was digested with restriction enzymes BamHI and Ncol from the human β-catenin PCDNA3.1 plasmid (Addgene, Watertown, MA, USA) and introduced into the lentiviral plasmid PCDHPCDH-CMV-EF1-copGFP-2A-Puro (Wuhan Institute of Virology, Wuhan, China). Next, site-directed mutagenesis was performed to replace the S246-P247 site of the β-catenin gene fragment in the lentiviral plasmid with 2 Xaa-P cis-peptides that had the highest and lowest ICPB. Finally, according to the method described in Yu et al. (27), these plasmids (Supplemental Fig. S1) were packaged into different lentiviral particles.

**Cell culture and screening for stable expression of β-catenin (X247-P)**

The culture method for the HepG2 cell line (an immortalized cell line consisting of human liver carcinoma cells; American Type Culture Collection, Manassas, VA, USA) is described in Yang et al. (28). When cells in 12-well plates reached 50–60%
confluence, the cells were transfected with the packaged viral particles indicated above using the method described in Joseph et al. (6). After 48 h, a screening of resistant cells was performed using culture medium containing 1 μg/ml puromycin (MilliporeSigma, Burlington, MA, USA). The culture medium was replaced every 3 d. The expression of the reporter gene copGFP from the lentiviral plasmid was observed under an inverted fluorescence microscope. When cell growth reached ~60% confluence and most cells expressed copGFP, cells were transferred into a 100-mm culture plate with a coverslips for expanded culturing. When the cell growth reached 60–80% confluence on the culture plate, transfected cells from all groups were collected for the interaction detection, and the coverslips with cells were used for subcellular localization tests.

Detection of interactions using coimmunoprecipitation

Transfected cells were scraped off the culture plates and ~10^7 cells were transferred to corresponding marked tubes and placed on ice. Cells were washed twice with ice-cold PBS before being centrifuged at 800 g for 5 min, and the supernatant was discarded. Then, the cells were resuspended in 500 μl of NP40 buffer (MilliporeSigma) and lysed on ice for 30 min. A total of 2000 μg of protein in 500 μl was used for immunoprecipitation. Next, a rabbit (D6W5B) anti-Flag mAb (CST, Shanghai, China) was added to determine the specificity of APC and E-cadherin, respectively. The anti-APC (Abcam, Cambridge, MA, USA) and anti-E-cadherin (CST) antibodies were used to determine the specificity of APC and E-cadherin, respectively. The β-catenin (S246-P247)-Flag fusion protein was used as the wild-type control, and the detailed method is described in Maguire et al. (24).

Subcellular localization detection using immunofluorescence

The coverslips with HepG2 cells were removed and dried after washing 3 times with PBS; the cells were fixed with 4% formaldehyde at room temperature for 15 min. The fixed cells were rinsed 3 times with 1× PBS for 5 min each. Normal goat serum was used to block the cells at room temperature for 60 min. After the blocking buffer, an anti-Flag-tag mouse mAb (1b10) (MilliporeSigma) at a dilution of 1:2000 was added to the cells and then incubated at 4°C overnight. The cells were rinsed with 1× PBS 3 times for 5 min each; then, diluted fluorescent material was added to stain the secondary antibody [anti-mouse IgG (H + L) (AbFluor 647 conjugated); MilliporeSigma]. The specimens were incubated in the dark for 2 h at room temperature. The cells were rinsed with 1× PBS 3 times for 5 min each. After dilution, DAPI was added, and the specimens were incubated in the dark for 10 min at room temperature. The cells were rinsed with 1× PBS 3 times for 5 min each; the slides were covered with coverslips and sealed at room temperature overnight. Finally, the specimens were observed under a fluorescence microscope.

RESULTS

Statistical analysis of cis- and trans-peptide bonds in the data set

There were 313,206 peptide bonds in the established nonredundant data set, which contained 1614 proteins. The statistical results showed that there were 312,160 trans-peptide bonds, which accounted for 99.67% of the total peptide bonds. There were 1046 cis-peptide bonds, which accounted for 0.334% of the total peptide bonds. Among the cis-peptide bonds, 759 Xaa-P cis-peptide bonds accounted for 72.6% (759/1046) of the total cis-peptide bonds. There were 287 Xaa-XnP cis-peptide bonds, which accounted for 27.4% (287/1046) of the total cis-peptide bonds. In our data set, there were 15,873 Xaa-P peptide bonds. Among these Xaa-P peptide bonds, the ICPB was 5% (759/15,837). The results are shown in Fig. 1.

Statistical analysis of the distribution of Xaa-P cis-peptide bonds and the ICPB in the data set

The ICPB for any 1 peptide bond is not only related to the number of its cis-peptide bonds but also related to the abundance of the peptide bond in proteins. To measure the ICPB of cis-peptide bonds in the data set, we analyzed differences in the abundance of Xaa in Xaa-P cis-peptide bonds in proteins in our nonredundant data set. The ICPB of 20 types of amino acids and proline were analyzed, and the results are shown in Fig. 2.

Figure 2 shows that the numbers of different Xaa-P cis-peptide bonds were different. When the Xaa of a cis-peptide bond was Y, W, G, E, F, S, or P, there was a greater tendency to form a cis-peptide bond. When the

Figure 1. The distribution of cis-peptide bonds in the nonredundant data set. A) A total of 312,160 trans-peptide bonds accounted for 99.67% of the total peptide bonds. B) There were 759 Xaa-P cis-peptide bonds accounting for 72.6% (759/1046) of the total cis-peptide bonds. There were 287 Xaa-XnP cis-peptide bonds, which accounted for 27.4% (287/1046) of the total cis-peptide bonds. C) In the data set, there were 15,873 Xaa-P peptide bonds. Among these Xaa-P peptide bonds, the ICPB was 5% (759/15,837).
Xaa was M, L, Q, V, I, H, T, or D, there was a greater tendency to form a trans-peptide bond. 

Survey of the amino acid residues surrounding each cis-peptide bond using the z-score method

To further investigate the mechanism underlying the development of cis-peptide bonds, the z-score method was used to analyze the fragments of 5 residues that were located before and after cis-peptide bonds in our data set, and the results are shown in Supplemental Table S1. Larger absolute z-score values indicated that they had greater effects on the formation of cis-peptide bonds and vice versa. To clearly observe amino acid residues that had greater effects, the residues that had larger effects on the formation of cis-peptide bonds were further analyzed, and the results are shown in Table 1.

The z-score analysis results indicated that sequence fragments with abundant A, G, L, P, S, or V before or after the peptide bond are favorable for forming cis-peptide bonds, and V at the P +5 position, L at the P +5 position, or P at the P +5 position were the most favorable for cis-peptide bond formation. When the upstream and downstream sequences (−5, +5) had abundant C, H, M, N, W, or Y, they were not favorable for X-P to form a cis-peptide bond, and W at the P +5 position, W at the P +5 position, and C at the P +5 position were the most unfavorable for cis-peptide bond formation.

Statistics for non-P cis-peptide bonds (Xaa-XnP)

To comprehensively understand the reasons for cis-peptide bond formation, the incidence of non-Pro cis-peptide bonds (Xaa-XnP) in the data set was analyzed according to the definition of the ICPB in this study. The results indicated that there were very few cis-peptide bonds in Xaa-XnP, with only 152 cis-peptide bonds distributed in 102 different types of peptide bond structures (a total of 380 types of Xaa-XnP). The ICPB for G-associated cis-peptide bonds (Xaa-G and G-Xaa) were the highest (0.213 and 0.18%, respectively). The ICPB for W-associated cis-peptide bonds (Xaa-W) was 0.118%. In addition, the ICPB for

| Residue/location | Conductive to the formation of the cis-conformation | Not conductive to the formation of the cis-conformation |
|------------------|---------------------------------------------------|-------------------------------------------------------|
| A +2             | C +5, C +4, C −2, C −5                            |
| G −1, G −2      | H +5, H −5, H +3                                 |
| L +5, L +4, L −3, L −4, L −5, L −2, L +2 | M +5, M +2, M −3, M −4, M −1, M +4, M +3, M −5 |
| P +5, P +3, P +2 | N+5, N+4                                          |
| S −3, S −1, S +5 | W +5, W −2, W +2, W +4, W +3, W −3, W +3, W −4  |
| V +5, V −5, V −4, V +4 | Y +5                                                |

Residues that have a strong influence on the ICPB (z > 1.5, z < −1.2). Fragments with abundant A, G, L, P, S, or V before or after the Xaa-P cis-peptide bond, especially V at the P +5 position, L at the P +5 position, and P at the P +5 position, have high z scores. However, fragments with abundant C, H, M, N, W, or Y before or after the Xaa-P trans-peptide bond, especially W at the P +5 position, W at the P +5 position, and C at the P +5 position, have z scores < 0.
P-W, W-A, I-I, and G-G were all higher than 0.05%; a cis-peptide bond distribution was not discovered in the 278 types of Xaa-XnP structures, such as A-D and A-E (Table 2).

**Screening and purification of the stable expression of β-catenin (X247-P)**

To ensure that the experimental results were not affected by the efficiency of gene transfection, 5 different lentiviruses containing β-catenin (Xaa246-P247) mutants were used to transfect the HepG2 cell line. In addition, HepG2 cells that stably expressed the 5 different β-catenin (Xaa246) mutants were screened using culture medium containing puromycin. The expression of copGFP was observed under an inverted fluorescence microscope (shown in Fig. 3).

**Detection of the effects of β-catenin (Xaa246-P247) mutants on interactions with APC and E-cadherin using co-IP**

To further validate the effects of different ICPB on protein functions, the proteins were extracted from HepG2 cell lines stably expressing 5 different types of β-catenin (Xaa246). Next, co-IP was performed to detect the interaction between β-catenin (Xaa246) with different ICPB and APC and E-cadherin. The results indicated that β-catenin (Y246) and β-catenin (W246), which had higher ICPB, had weaker interactions with APC (shown in Fig. 4A) and E-cadherin (shown in Fig. 4B); β-catenin (L246) and β-catenin (M246), which had lower ICPB, had stronger interactions with APC (shown in Fig. 4A) and E-cadherin (shown in Fig. 4B).

**Cellular localization detection**

Immunofluorescence was used to detect the nuclear localization of the β-catenin (Xaa246) mutants with different ICPB. For immunofluorescence tests, the β-catenin (Xaa246-P247) mutants were stained red by anti-Flag conjugated with Fluor 647, and the nuclei were stained in blue. The results indicated that β-catenin (L246) and β-catenin (M246), which had lower ICPB, had less nuclear localization, whereas β-catenin (Y246) and β-catenin (W246), which had higher ICPB, had more nuclear localization (Fig. 5).

**DISCUSSION**

**The definitions of cis-peptide bonds**

The earliest study of bond angles hypothesized that the 6 groups that form the peptide bond in the protein polypeptide chain are on the same plane, so peptide bonds with a 0° ω dihedral angle were defined as cis-peptide bonds, and peptide bonds with a 180° ω dihedral angle were defined as trans-peptide bonds (2). Subsequent studies showed that ω values equal to 0° and 180° rarely occurred. Therefore, cis-peptide bonds were defined as ω values equal to 0 ± 30°, and trans-peptide bonds were defined as ω values equal to 180 ± 30°. Cis-peptide bonds in the PDB were defined using this method. During statistical analysis using atomic coordinates in the PDB, we found that the ω values of many peptide bonds were 30° < ω < 90° and 90° < ω < 150°, except for the distribution in the ranges of 0 ± 30° and 150 ± 30°. These results suggested that many cis-peptide bonds were not counted in the cis-peptide records PDB file (CISPEP) document of the PDB according to the nomenclature principle of the cis-trans isomerism. Therefore, in this study, we defined cis-peptide bonds as 0° < ω < 90° and trans-peptide bonds as 90° < ω < 180°.

**The association between proline structure and the ICPB**

Joseph et al. (6) noted that the majority of peptide bonds in polypeptide chains show the trans-conformation. Very few peptide bonds show the cis-conformation, and they are mainly Pro-containing peptide bonds. The major reason is that the steric hindrance induced by the tetrahydropyrrole ring of proline eliminates the advantage of the trans-conformation, so it is relatively easy for the Xaa-P peptide bond to span the potential barrier between 2 conformations and form a cis-peptide bond (29). In addition, Lu et al. (30) showed that the energy difference between the cis- and trans-conformational bonds of general peptide bonds is 2.5 kcal/mol, and the rotation energy barrier is 20 kcal/mol. Meanwhile, these values are 0.5 and 13 kcal/mol, respectively, for the peptidyl-prolyl imide bond. These results suggested that the presence of proline reduces the energy barrier of the conversion between cis- and trans-peptide bonds, which reduces the double bond property of peptide bonds and makes the conversion of trans-peptide bonds to cis-peptide bonds easier. In our results, we found that the majority of cis-peptide bonds are found in Xaa-P peptide bonds, which accounted for 72.6% of the total cis-peptide bonds, whereas non-proline-associated cis-peptide bonds (Xaa-XnP) accounted for only 27.4% (Fig. 1). Moreover, our results also showed that the occurrence of cis-peptide bonds is mainly related to the special structure (tetrahydropyrrole ring) of proline in peptide bonds.

**The effects of different Xaas on the ICPB of Xaa-P peptide bonds**

Wu et al. (31) considered that the interaction between the flanking aromatic rings of Y, W, and F residues in the GXPG short peptide and the pyrrole ring of proline can maintain a stable conformation of the Xaa-P cis-peptide bond. In this study, we found that both Y-P and W-P had higher ICPB—which were 12.2 and 8.74%, respectively—and were much higher than the 5% mean

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The first line shows the XnP for 19 aa other than proline in Xaa-XnP cis-peptide bonds. The first column shows Xaa (20 aa) in Xaa-XnP cis-peptide bonds. The numbers indicate the ICPB cross between the lines and columns. The right column shows the mean ICPB on the respective line, and the bottom line shows the mean ICPB in the respective column. Each number is rounded to 4 digits. In our data set, there were very few cis-peptide bonds distributed in 102 different types of peptide bond structures (a total of 380 types of Xaa-XnP). The G-associated ICPB (Xaa-G and G-Xaa) were the highest (0.213 and 0.18%, respectively). The W-associated ICPB (Xaa-W) was 0.11%. In addition, the ICPB for F-W, W-A, H-I, and G-G were all higher than 0.05%, whereas the distribution of cis-peptide bonds was not found in the other 278 types of Xaa-XnP structures, such as AlaAsp and AlaGlu. *Peptide bond with a high ICPB value.
value (Fig. 2). In contrast to other studies, the G-P peptide bond also had a higher ICPB. This higher incidence is possibly due to the special molecular structure of G. Among the 20 types of amino acids that constitute human proteins, G has no flanking R group but has a hydrogen atom in its structure, so the steric hindrance between the G-P trans-peptide bond and G-P cis-peptide bond is smaller than that of other peptide bonds, and the G-P trans-peptide bond is easier to convert to a G-P cis-peptide bond. Thus, in our results, the ICPB in G-P was 9.12% and ranked second among the 20 aa after only Y-P (Fig. 2).

Figure 3. Screening and purification of the stable expression of β-catenin (X246-P247). To obtain stable expression of β-catenin in cells, HepG2 cells were screened using puromycin medium 144 h after lentiviral transfection, and all cells were observed under a fluorescence microscope (4 ×10): M246-P247 (A); L246-P247 (B); W246-P247 (C); Y246-P247 (D); and S246-P247 (E).

Figure 4. Detection of interactions: co-IP was used to detect the interactions of β-catenin (Xaa246-P247) mutants with APC and E-cadherin. A) β-Catenin Y246 and W246, which had higher ICPB and weaker interactions with APC; β-catenin L246 and M246, which had lower ICPB, had stronger interactions with APC; B) β-Catenin Y246 and W246, which had higher ICPB, had weaker interactions with E-cadherin; β-catenin L246 and M246, which had lower ICPB, had stronger interactions with E-cadherin. IgG H.C., positive control; IP, immunoprecipitation.
The relationship between the ICPB and the types of Xaa in fragments before and after Xaa-P peptide bonds

The above discussion showed that the single-amino-acid residue Xaa before the proline residue had important effects on the occurrence of \(\text{cis}\)-peptide bonds. In addition, studies from Joseph et al. (6) and Exarchos et al. (25) indicated that the occurrence of Xaa-P \(\text{cis}\)-peptide bonds is associated with features of the fragment before and after Xaa-P. To further investigate the effects of flanking amino acid residues on the conformation of Xaa-P peptide bonds, we analyzed the \(z\) scores of proline residues at all positions. The results indicated that some amino acids at specific positions had a certain association with \(\text{cis}\)-peptide bonds. When the fragments before and after the peptide bond (−5, +5) had abundant G, L, P, S, A, E, or V, conditions were favorable for forming Xaa-P \(\text{cis}\)-peptide bonds (Table 1).

ICPB of \(\beta\)-catenin (X246-P247) in HepG2 cells

The protein structures in the PDB were mainly determined from the prokaryotic cell expression system. Because of the influences of different environmental factors, such as the post-translational modification system and molecular chaperones (32, 33), the protein structures obtained from X-ray analysis might be different from those in the environment of human tissues and mammalian cells. Therefore, protein structural data obtained from technologies such as X-ray analysis still need to be validated in animal cells. Thus, in this study, a statistical approach, Co-IP, and immunofluorescence were used to confirm that S246→Y and S246→W missense mutations, which help increase the ICPB in Xaa246-P247 in human \(\beta\)-catenin, can reduce the interaction between \(\beta\)-catenin and APC (Fig. 4A) and between \(\beta\)-catenin and E-cadherin (Fig. 4B), eventually leading to increased nuclear migration of \(\beta\)-catenin in HepG2 cells (Fig. 5).
Generally, these conclusions need experimental results to confirm that the amino acid substitutions performed indeed promote either cis- or trans-configurations as suggested from the statistical data [e.g., by NMR analysis of peptides, such as in Wu and Raleigh (31)]. However, the NMR method in Wu and Raleigh (31) is only applicable to the conformational determination of small molecular peptides, and the MW of β-catenin is too large and affected by spectral peak overlap to be used to determine conformational changes in peptide bonds of β-catenin (X246-P247).

Is β-catenin (S246) involved in binding to APC?

If β-catenin (S246) is involved in binding APC, the mutations might directly interfere with APC interactions. In that case, it would be difficult to distinguish whether the effects observed are due to the cis- vs. trans-configuration because mutations alter APC binding sites. If S246 is involved in binding APC, then the interaction strength between β-catenin and APC will be weakened or absent, regardless of whether S246 was mutated to any other amino acids (except possibly Y or T); however, our experiments show that the interaction was strengthened when S246 was mutated to M or L, whereas the interaction was weakened when S246 was mutated to Y or W (Fig. 4A). This result indicates that S246 is not involved in binding to APC.

The results indicated that local ICPB changes in β-catenin (S246-P247) affected the interaction between β-catenin and APC molecules and eventually resulted in changes in the distribution of β-catenin in the nucleus and cytoplasm (Fig. 5) and further affected the expression of downstream genes of the Wnt–β-catenin signaling pathway.

CONCLUSIONS

In most cases, Xaa-P has the highest ICPB when cis-peptide bonds are distributed in the Xaa-P segment of the polypeptide chain, especially when Xaa is Y, W, F, or G. Alterations in the ICPB at the β-catenin (Xaa246-P247) site eventually resulted in changes in the subcellular localization of β-catenin and interactions with APC and E-cadherin.

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AUTHOR CONTRIBUTIONS

S. Yu performed experiments, collected and assembled data, and wrote the manuscript; Y. Yang conceptualized and designed the experiments, wrote the manuscript, and gave financial support and administrative support; Y. Zhang and H. Yang collected data and performed the experiments; Y. Wu and Y. Chen performed major experiments; and Z. Zhang gave administrative support and final approval of manuscript.

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