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Mechanisms of PDGFRalpha promiscuity and PDGFRbeta specificity in association with PDGFB

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1. ABSTRACT

Platelet-derived growth factor receptor alpha (PDGFRalpha) interacts with PDGFs A, B, C and AB, while PDGFRbeta binds to PDGFs B and D, thus suggesting that PDGFRalpha is more promiscuous than PDGFRbeta. The structural analysis of PDGFRalpha-PDGFA and PDGFRalpha-PDGFB complexes, and a molecular explanation for the promiscuity of PDGFRalpha and the specificity of PDGFRbeta remain unclear. In the present study, we modeled the three extracellular domains of PDGFRalpha using a previous crystallographic structure of PDGFRbeta as a template. Additionally, we analyzed the interacting residues of PDGFRalpha-PDGFA and PDGFRalpha-PDGFB complexes using docking simulations. The validation of the resulting complexes was evaluated by molecular dynamics simulations. Our results show that that changes of non-aromatic amino acids in PDGFRalpha to aromatic amino acids in PDGFRbeta (I139F, P267F and N204Y) may be involved in the promiscuity of PDGFRalpha. These results may be used as an input for a better peptide design targeting diseases related with the malfunction of PDGF system such as cancer and atherosclerosis.

2. INTRODUCTION

Platelet-derived growth factors (PDGFs A, B, C, and D) are the key mitogens for connective tissue cells like smooth muscle cells and fibroblasts, and enhance wound healing and maintain connective tissue homeostasis in adulthood (1,2) and critically regulate embryonic development (3-6). On the other hand, healing process mediated by PDGF overexpression can occur due to inflammation or chronic injury that leads to fibrosis of pathological tissues (7). Previous studies reported that aberrant expressions of PDGF and its receptor (PDGFR) are often associated with a variety of diseases including atherosclerosis, cancer, fibroproliferative diseases of lungs, kidneys and joints (8,9).

PDGF as a mitogen is composed of a family of five dimeric PDGF ligands, PDGF's A, B, C, D, and AB with two tyrosine kinase receptors, the PDGF alpha and beta (Ralpha and Rbeta) (10). The A and B chains of PDGFs are synthesized as precursors and undergo proteolytic processing after dimerization. Ralpha and Rbeta possess 60% amino acid identity, with eight conserved cysteine residues (11). Ralpha promiscuity may rely on its ability to bind PDGF's A, B, and C (12), whereas Rbeta might

have specificity for PDGFs B and D (13). Although there are functional evidences for the homodimeric complexes of PDGFA-Ralpha, PDGFC-Ralpha, and PDGFB-Rbeta, some biochemical data support additional homodimeric and heterodimeric combinations (14). In such biological context, PDGFB is produced by almost all types of solid tumors, and PDGFR signaling participates in various processes including stimulation of tumor angiogenesis, recruitment of tumor stroma fibroblasts, and autocrine stimulation of tumor cell growth (2). In recent years, blockade of PDGFRs signaling has become an efficient therapeutic strategy against cancer (15), and a combined inhibition of vascular endothelial growth factor (VEGF) and PDGF has emerged as a promising therapy for suppressing angiogenesis in tumor progression (16).

The X-ray crystal structures of PDGFA and its propeptide, and PDGFB-Rbeta complexes have been reported previously (17). However, the structural analysis of complexes Ralpha-PDGFA and Ralpha-PDGFB are unknown due to the lack of suitable crystal structure and difficulties of protein multimerization in solution (17). Moreover, other important aspects in the interaction of complexes Ralpha-PDGFA and Ralpha-PDGFB remain unclear. Elucidating these interactions may shed light on the promiscuity and specificity related mechanisms of Ralpha and Rbeta, respectively. In this research article, protein-protein docking simulations, molecular modeling and docking validation by molecular dynamics simulation were assessed to predict the structure of Ralpha and its interaction with PDGFAXY and PDGFBXY (X and Y represent each chain). Furthermore, we analyzed the residues involved in the interaction in Ralpha-PDGFB and Rbeta-PDGFB complexes, and hypothesize possible mechanisms of Ralpha promiscuity and Rbeta specificity.

3. MATERIALS AND METHODS

3.1. Sequence Alignment

The primary sequence of the human Ralpha protein was obtained from the GenBank database (Accession: AAH63414). BLAST (18) was used to search suitable templates in RCSB Protein Data Bank (PDB) for the Ralpha sequence. Multiple sequence alignment (MSA) with Ralpha, Rbeta, VEGFR1 and VEGFR2 was performed by Muscle (19). For MSA, we chose VEGFR1 (Accession: P17948) and VEGFR2 (Accession: P35968), as both shared some similarities with PDGFRs (20).

3.2. Molecular modeling and validation

The molecular modeling experiments were executed under Molecular Operating Environment (MOE) using AMBER99 force field (21). The predicted homology model was generated using the X chain from the PDGFRbeta-PDGFB (PDB ID: 3MJG) crystal structure. Moreover, other ten intermediate models were generated and their Cartesian average was taken as the final model. The minimizations were performed with MOE until it reaches a RMSD gradient of 0.0.5 kcal mol-1Å-1. Stereochemical quality of the polypeptide backbone and side chains were evaluated using Ramachandran plot (22), and the global score validation was calculated with QMEAN6 (23).

3.3. Protein-protein docking simulation

The crystal structures of PDGFs A and B were retrieved from PDB database (PDB ID: 3MJK; (17,24). The structure of PDGFA in complex with its propeptide (PDB ID: 3MJK) and Rbeta in complex with PDGFB (PDB ID: 3MJG) were chosen to assess the interacting residues for PDGFA (Y157-K160 and N134-V138) and for PDGFB (I76-K80 and R32-N36) to perform docking simulations in the ZDOCK (25) and ClusPro (26) programs. The candidate residues on the surface of interacting proteins and the experimental information on PDGFA-propeptide and Rbeta-PDGFB complexes reported previously were used to filter the docking results (17).

3.4. Docking validation by molecular dynamics simulation

The complexes Ralpha-PDGFB and Ralpha-PDGFA obtained from docking analyses were subjected to MD simulation (performed by GROMACS package v.4.5.5.) studies to refine the protein interface (27). The two complexes were inserted into SPC solvated cubic box, and the system was neutralized by the addition of Cl- at random box, and the system was neutralized by adding Cl- at random positions in the solvent. The simulations were performed using the AMBER99 force field. Energy minimization was carried out by assessing the steepest descent method during 5000 steps with GROMACS program. MD stimulations were performed at constant pressure (1 atm) and temperature (300 K) using the Berendsen algorithm. The equilibration stage was performed with all atomic protein positions restrained during 100 ps, followed by a simulation without restraint for 10 ns. The time step used for all simulation was 2-fs (28,29). The final conformation was used to compute inter-residue distances, identify specific interactions at the interface and other calculations.

3.5. Docking interface analysis

Protein-protein interactions were analyzed with LigPlot+ v1.4. (30), using the DIMPLOT program for protein-protein interactions. This program shows hydrophobic and hydrogens bond interaction between proteins. Electrostatic potential surface and interaction of the complexes were calculated and represented by using Pymol v1.5.

4. RESULTS

We performed a search against PDB database to find suitable templates for Ralpha. Rbeta was chosen

as template to model the 3D structure of the Ralpha. The BLAST results showed a 38% coverage, 32% identity and E-value of 9e-44. This 32% identity corresponded to the 3 extracellular domains (from the 23 AA to 306 AA in Ralpha), involved in the interactions with PDGFA and PDGFB. The RMSD of Ralpha and Rbeta showed low differences between the two receptors (0.5.9 Å; Figure 1). The predicted 3D structure of Ralpha had 3 I-set Ig-like domains (D1, D2, and D3), where the binding site of the PDGFs was limited to the D2 and D3 domain (Figure 2). Stereochemical quality of the built model indicated that 80.4.% of the residues lied in most favored regions and 0.4.% of the residues were in disallowed regions. The predicted model showed a global structural score of 0.6.29 and a Z-score of -1.5.3; these values suggested a correct fold of the modeled protein.

4.1. Protein–protein docking

The predicted model of Ralpha was used in a docking simulation between PDGFA (PDB code: 3MJK) and PDGFB (PDB code: 3MJG). To include only biologically relevant structures, experimental data were used from previous reported structures of Rbeta-PDGFB and PDGFA-propeptide (17). The final docked model was also selected based on factors such as the area of surface contact, extent of interactions and stability of the model. Furthermore, the most favorable docking solution between Ralpha-PDGFA and Ralpha-PDGFB complexes were refined by MD simulation, and the structural stability of both complexes was investigated based on the root mean square deviation (RMSD). The RMSD of the backbone in Ralpha-PDGFA complex increased from 0 Å at 0 ns to ~0.5. Å at 6.5. ns, but it remained stable after 6.5. ns (Figure 3A). On the other hand, Ralpha-PDGFB complex reached stability after 3.5. ns (Figure 3B). Since complexes reached a certain structural stability at 10ns, the final conformation of two complexes in the MD simulation was selected as the final docked structure (Figure 4). Moreover, electrostatic potential comparison of the PDGFA with Ralpha, and PDGFB with Ralpha was performed to validate whether the two complexes were electrostatically complementary in the binding area. Our results showed that the PDGFRalpha, between D2 and D3 regions, had strong negative charges (+70.7.60 kT/e and -70.7.60 kT/e; Figure 2B) and might be electrostatically complementary to PDGFA (+67.7.96 kT/e and -67.7.96 kT/e) and PDGFB (+68.4.57 kT/e and -68.4.57 kT/e) due to the positive charges in the interacting region with Ralpha (Figure 5).

The protein-protein complexes (Ralpha-PDGFA and Ralpha-PDGFB) showed that interactions were mostly hydrophobic due to the number of non-polar amino acids in the binding area such as Y, V, F, M, I, G and L (Tables 1 and 2). Some of these amino acids were conserved in Ralpha when interacted with PDGFA and PDGFB, and might play a key role in the hydrophobic interactions (V 155, V 242, E 241 and Y 273). Finally, the docking results were analyzed and compared with experimental data obtained from a previous study, where the 3D structure of PDGFB-Rbeta and PDGFA-propeptide complexes were reported. These comparisons were performed by using MSA (Figure 6).

4.2. PDGFA-Ralpha complex: interface description

Complex of PDGFA with Ralpha showed that the chains PDGFAX and PDGFAY had hydrogen bond interactions with Ralpha monomer. Nevertheless, the binding residues of both monomers in each binding site were different when in complex with Ralpha. Specifically, the residues implicated in the complex PDGFAX-Ralpha were Y 157, R 159 and N 116 (for chain X) and D 244, E 262, T 296, N 240 and V 243 (for receptor alpha; Figure 7A). As for PDGFY-Ralpha binding, the residues were S 114, N 116, K 160, K 161 and R 159 (for chain Y) and E 262, A 295, E 298 and D 244 (for receptor alpha; Figure 7B).

Structural analysis to assess possible hydrophobic interaction between Ralpha and PDGFA showed that a core of hydrophobic amino acid led the interaction in this complex. Moreover, the residues implicated in the complex PDGFAX- Ralpha were V 155, L 118, F 117 and V 158 (for chain X) and V 184, I 264, V 242, V 243, V 299, M 260 and M 133 (for receptor alpha; Figure 8A). Regarding PDGFAY-Ralpha binding, the hydrophobic core was composed by L 118, F 117, V 158 and V 155 (for chain Y) and V 184, V 266, V 219, M 260, L 261, L 245, L 137 and I 139 (for receptor alpha; Figure 8B; for a complete list see Table 1).

4.3. PDGB-Ralpha complex: interface description

The protein-protein interactions of this complex were mostly hydrophobic, but presented some hydrogen bond interactions as well. The residues implicated in the complex PDGFBX-Ralpha were R 79, K 80, R 27 and N 54 (for monomer X); and E 218, A 272, T 273 and V 182 (for receptor alpha; Figure 9A). Furthermore, in the PDGFBY-Ralpha binding, the residues were N 34, R 32, R 28, R 27, R 79, T 101 and R 56 (for monomer Y) and A 272, L 222, E 240, Y 183 and T 160 (for receptor alpha; Figure 9B).

Hydrophobic interactions in the PDGFB-Ralpha complex resembled to the pattern described with the PDGFA-Ralpha complex, where the binding region for the two proteins were rich in hydrophobic residues such as L, I, M, F and V. A general view of the hydrophobic interactions of the PDGFBX-Ralpha complex showed that the interacting residues were I 76, I 77, F 37, L 38 and F 84 (for monomer X) and L 222, M 110, L 138, M 137 and I 116 (for receptor alpha; Figure 10A). As for PDGFBY-Ralpha binding, the residues were F 84, L 38, I 77 and F 37 (for monomer Y) and M 237, I 116, L 238, M 110 and L 222 (for receptor alpha; Figure 10B; for a complete list see Table 2).

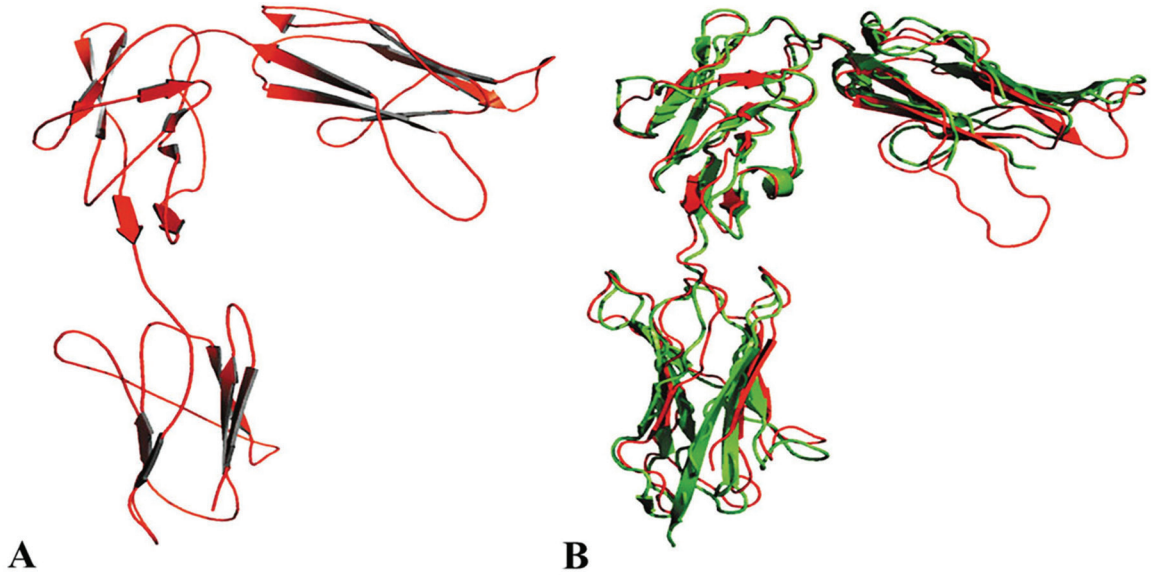

Figure 1. Structure of Ralpha by homology modeling performed in MOE: (a) Side view of the ribbon model; (b) The structure alignment between Ralpha and PDGFRbeta (3MJG) presented a RMSD 0.5.60 Å with modeled structure (in red) and template (in green), demonstrating that this model may be used in next analyses.

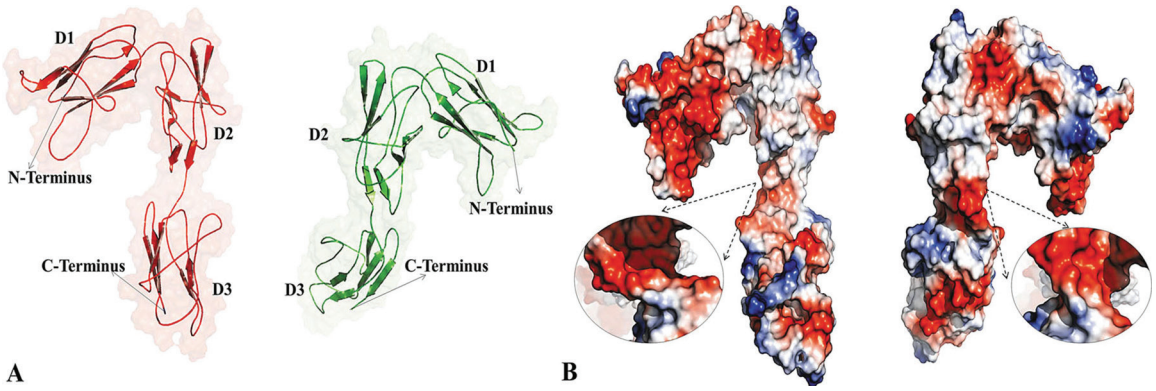

Figure 2. (a) Ralpha structure obtained by homology modeling performed with MOE: In red and green are the Ralpha monomers; (b) Electrostatic potential generated of Ralpha. The blue and red represent positive and negative charges, respectively (+70.7.60 kT/e and -70.7.60 kT/e electrostatic scale). The three extracellular domains (D1, D2 and D3) and the negative charge in the binding site of Ralpha (between D2 and D3) are noted in the inserts.

5. DISCUSSION

In the current study, we modeled Ralpha using Rbeta (PDB ID: 3MJG) as template and performed a docking simulation of Ralpha in complex with PDGFA, and in complex with PDGFB. Using experimental data obtained from the crystallographic structure of PDGFB-Rbeta complex and a MSA, we identified the residues in common or those that presented substitutions between Rbeta and Ralpha in the binding area with PDGFB (M133A, N163E and N179S). These substitutions in both receptors may suggest possible mechanisms of Ralpha promiscuity and Rbeta specificity.

Our results along with the complex of PDGFA and the propeptide showed that PDGFA shared a large group of residues interacting with Ralpha and propeptide (V155, T 157, R 159, N 116, E 156, L 118, P 162, S 137, T135, N134, E90 and K160). Since Rbeta does not bind PDGFA, the interacting residues in Ralpha-PDGFA complexes were not compared. Moreover, a previous mutagenesis study of PDGFA reported that R 159, K 161 and P 162 are important residues in the interaction with Ralpha (31), and these three amino acids are also present in the interaction with PDGFA propeptide. This suggest that these residues are conserved in the interaction of PDGFA and may play an important role in the binding of other receptors associated to Ralpha, such as Ralphabeta.

Concerning the PDGFB in complex with Ralpha and Rbeta, R 27 is considered an important residue in

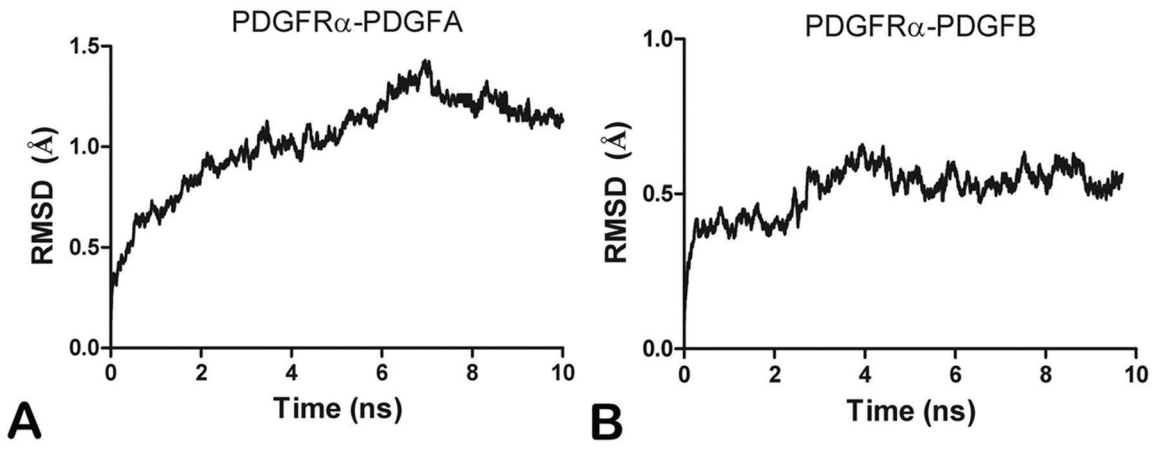

Figure 3. Graphical representation of root mean square deviation (RMSD) plot: RMSD for (a) Ralpha–PDGFA and (b) Ralpha–PDGFB from the initial structures throughout the simulation of 10 ns as function of time. Shows that the two complexes reach stability through the MD simulation.

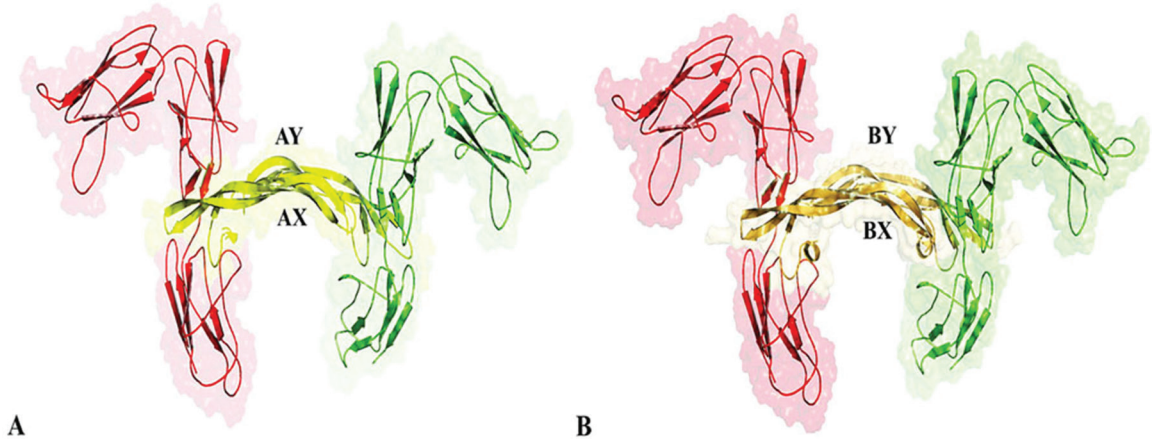

Figure 4. Representation of the Ralpha–PDGFA and B complexes obtained by docking and MD simulation. (a) Ralpha–PDGFA complex; PDGFA monomers are colored yellow, while Ralpha is colored red and green; (b) Ralpha–PDGFB complex; PDGFB monomers are colored light orange, while Ralpha is colored red and green. X and Y represents each chain of both PDGFA and PDGFB.

the interaction (32). We found that N 34, R 28, R 79 and N 54 are present in the hydrogen bond formation with the above mentioned receptors, suggesting that these residues may also be linked to R 27. On the other hand, the common residues found in these receptors that interact with PDGFB were Y206Y, E241E, V242V, Y273Y and V243V. This data suggests that these residues are conserved and may play a key role in the interaction with other mitogens.

Ralpha may be activated by PDGFA, PDGFB, PDGFC and the heterodimeric PDGF-AB. On the other hand, Rbeta can be only activated by PDGFB and PDGFD (33), suggesting that Ralpha is more promiscuous than Rbeta. It is important to clarify that Rbeta could bind other proteins such as phospholipase C gamma-1 and phosphoinositol-3-kinase (34, 35); nevertheless, in this paper, the specificity of Rbeta is based only in the interaction with PDGFs. Furthermore, since possible residues implicated in the interaction with PDGFB-Rbeta were previously reported (17), and using the structural model of PDGFB-Ralpha obtained from this study, we analyzed the residues substitution that in turn could explain the specificity and promiscuity of both receptors. Our results showed that non-aromatic amino acids in Ralpha are substituted to aromatic amino acids in Rbeta D2 and D3 binding sites (I139F, L245F, L137F, P267F and N204Y). This modification has a consequence in the torsion freedom of residues interactions, thus becoming more flexible in Ralpha compared to Rbeta. Although the two aromatic amino acids L137F and P267F are absent in the complex interaction of Rbeta-PDGFB, these residues could regulate the region around and may be less exposed and flexible in the unliganded state. This observation corroborates with a previous study, which hypothesized that substitution of non-aromatic to aromatic amino acids might explain the specificity of Rbeta (17).

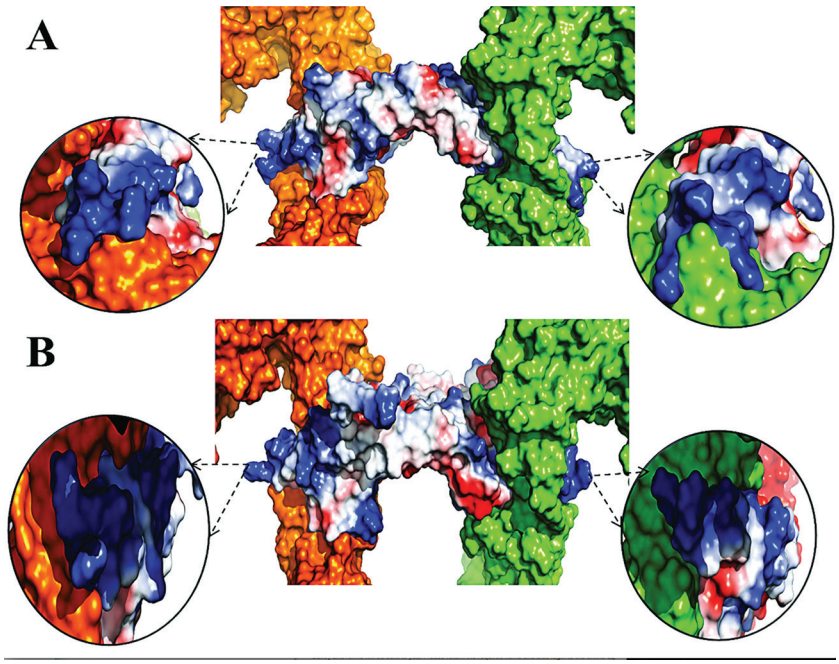

Figure 5. Electrostatic potential of (a) PDGFA and (b) PDGFB in complex with Ralpha: The blue and red indicate positive and negative charges, respectively. (a) Electrostatic scale (+67.7.96 kT/e and -67.7.96 kT/e) and (b) electrostatic scale (+68.4.57 kT/e and -68.4.57 kT/e). The blue colored structure represents the positive charge, where PDGFs interacts with Ralpha (in inserts).

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                                10        20        30        40        50
VEGFR1 HUMAN / 140-320  SEIPEIIHMTEGRE--LVIPCRVTSPNITVTL-KKFPLDTLIPDGKRIIW
VEGFR2 HUMAN / 130-316  SDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISW
PDGFRα HUMAN / 133-299  MTDY-LVIVEDDDS--AIIPCRTTDPETPVTL-HNSEG--VVP----ASY
PDGFRß HUMAN / 132-300  AEEL-FIFLTEITE--ITIPCRVTDPQLVVTL-HEKKGDVALP----VPY

                                60        70        80        90       100
VEGFR1 HUMAN / 140-320  DSRKGFIISNATYKEIGLLTCEATVNGHLYKTN-YLTHRQTNTI-IDVQI
VEGFR2 HUMAN / 130-316  DSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRI-YDVVL
PDGFRα HUMAN / 133-299  DSRQGFN----GTFTVGPYICEATVKGKKFQTIPFNVYALKATSELDLEM
PDGFRß HUMAN / 132-300  DHQRGFS----GIFEDRSYICKTTIGDREVDSDAYYVYRLQVSS-INVSV

                               110       120       130       140       150
VEGFR1 HUMAN / 140-320  STPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRA--SVRRRI
VEGFR2 HUMAN / 130-316  SPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLK
PDGFRα HUMAN / 133-299  EALKTV-YKSGETIVVTCAVFN--NEVVDLQWTYPGEVKGKGITMLEEIK
PDGFRß HUMAN / 132-300  NAVQTV-VRQGENITLMCIVIG--NEVVNFEWTYPRKESGR---LVEPVT

                               160       180       190
VEGFR1 HUMAN / 140-320  DQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFK
VEGFR2 HUMAN / 130-316  TQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTK
PDGFRα HUMAN / 133-299  VPSIKLVY----TLTVPEATVKDSGDYECAARQATREV
PDGFRß HUMAN / 132-300  DFLLDMPYHIRSILHIPSAELEDSGTYTCNVTESVNDH
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Figure 6. Multiple sequence alignment of PDGFRs binding areas, with VGFR1 and VGFR2 included for comparison: In yellow are the residues changes of non-aromatics to aromatics amino acids in Rbeta. In blue are the residues with side chains able to form >4 hydrogen bond. In orange are the residues changes with a high to low probability of rotamer changes in Rbeta.

Hydrophobic interactions play an important role in the recognition of PDGFRs to PDGFs, but hydrogen bond formations are also significant in the specificity of protein-protein interactions (36). Ralpha and Rbeta with PDGFB association are strongly affected by electrostatic interaction (Figure 5B), and this condition is important in the formation of hydrogen bonds (37). Ralpha forms eleven hydrogen bonds, while Rbeta forms fifteen hydrogen bonds when in complex with PDGFB. These outnumbered hydrogen bonds may influence the energy stability of the complex, as this hydrogen number differences may provide an extra free energy to strengthen this binding (38). Nevertheless, an unfilled hydrogen bond donor/acceptor may destabilize the binding and avoid the interactions (37) with others PDGFs. This is more likely to occur in Rbeta, as this

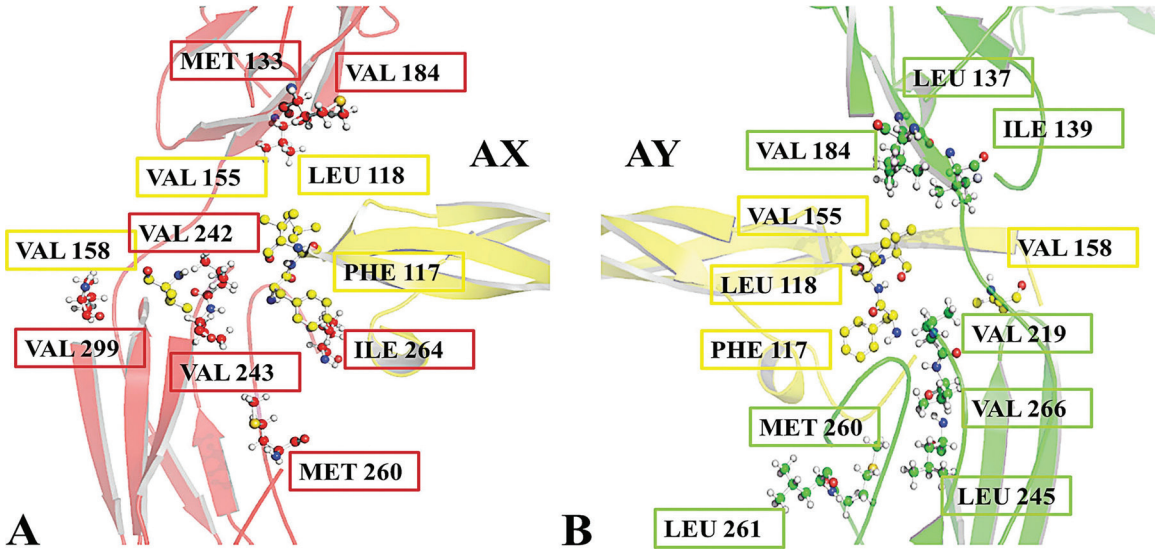

Figure 7. The PDGFA-Ralpha hydrogen bond interface analysis was performed with DIMPLOT: The residues involved in the binding site are shown as balls and sticks. (a) PDGFAX hydrogen bond formation with Ralpha monomer (red) and (b) PDGFAY hydrogen bond formation with Ralpha monomer (green). In contrast, the chains, PDGFBX and PDGFBY, do not form hydrogen bonds.

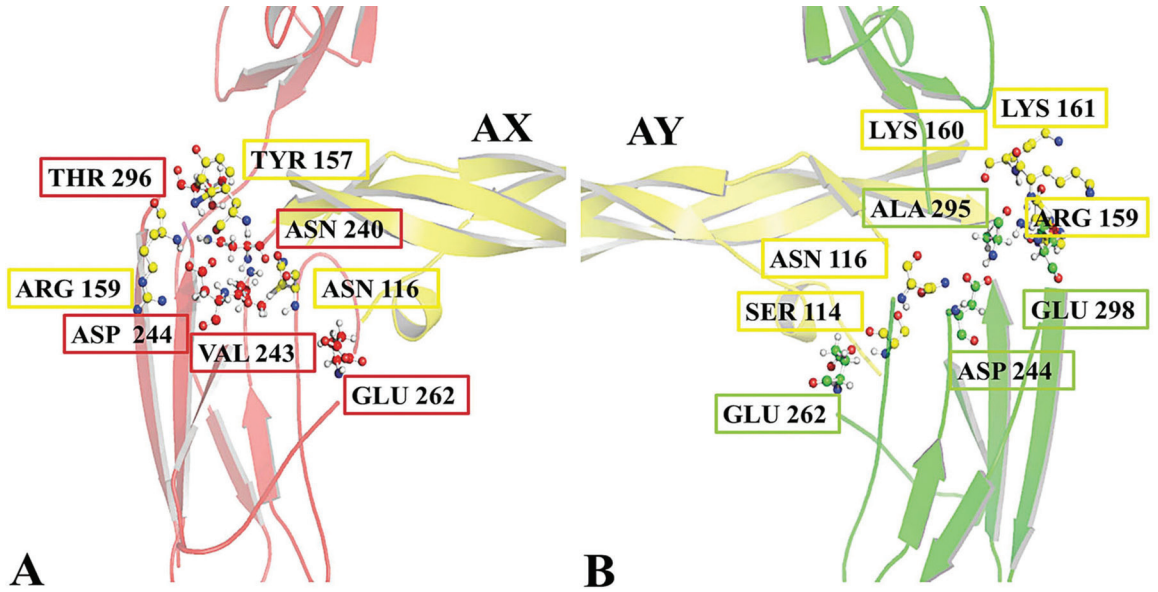

Figure 8. Hydrophobic core of PDGFA-Ralpha complex analysis was carried out with DIMLOT: The residues involved in the hydrophobic interactions are represented as balls and sticks. (a) PDGFAX interaction with the monomer of Ralpha (red); (b) PDGFAY binds the monomer of Ralpha (green). Despite these observations, the chains, PDGFAX and PDGFAY are not able to form hydrophobic core.

receptor presents R186, D185, N244 and E241 residues, thus representing a higher number of amino acids in its structure when compared to Ralpha (E241 and E 262).

A cluster of common amino acids (M133A, N163E, N179S, K194G, K197E, M218V, N239G, Q246E, K254S, K265T, K283E, M260L, R293T and Q294E) are more likely to suffer rotamer changes in PDGFalpha in comparison to Rbeta (30-50% and 0-20%, respectively (39,40). These amino acid substitutions suggest that Ralpha is more able to interact with other proteins. The substitution of non-aromatic amino acids in Ralpha and energy stability of Rbeta may explain why Ralpha interacts with various PDGFs when compared to Rbeta, which only binds PDGFB and PDGFD.

The novel information here is the complete characterization of interactions between Ralpha with

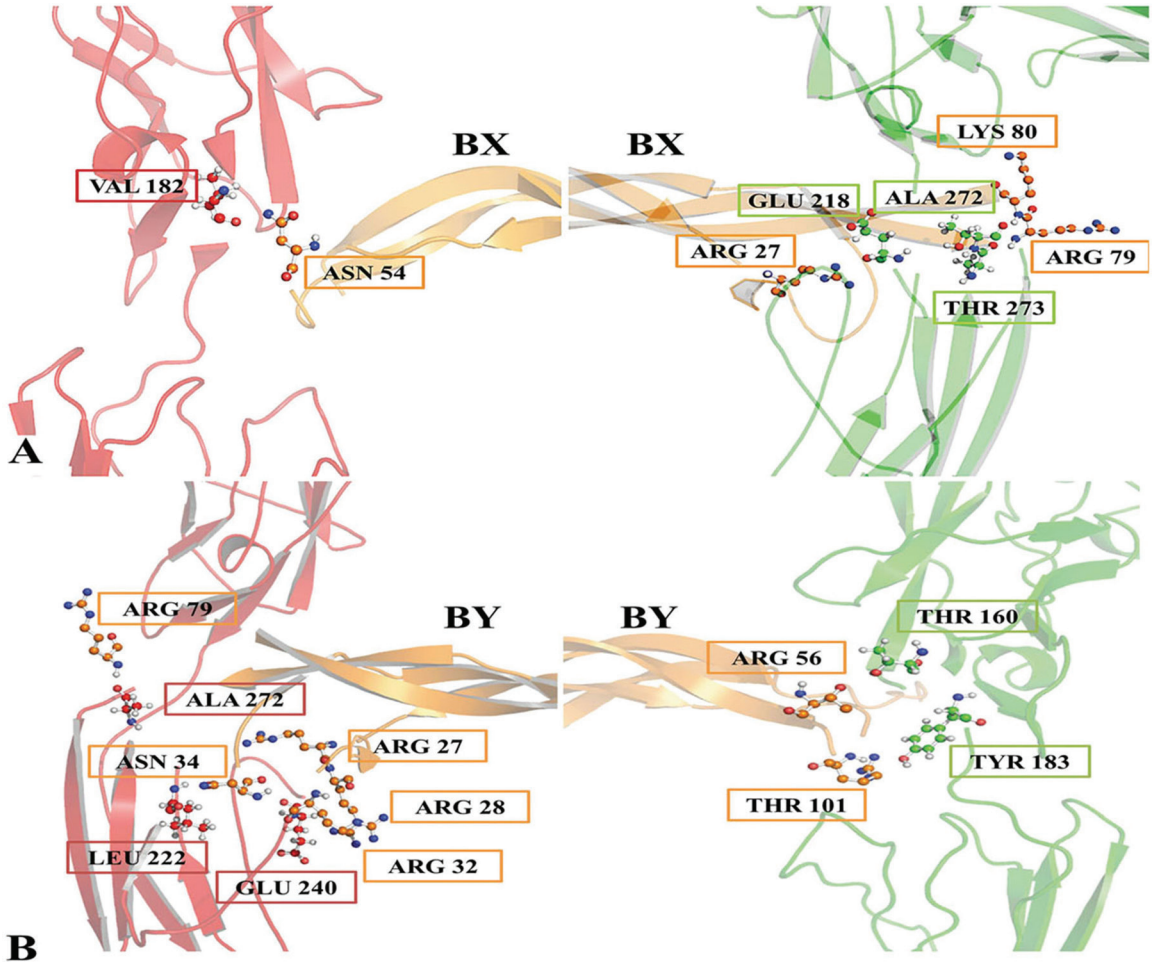

Figure 9. The PDGFB-Ralpha hydrogen bond interface analysis was performed with DIMLOT: The residues involved in the binding are shown as balls and sticks. (a) PDGFBX forms a complex with PDGFRalpha monomers (red and green); (b) PDGFBY binds Ralpha monomer (red and green).

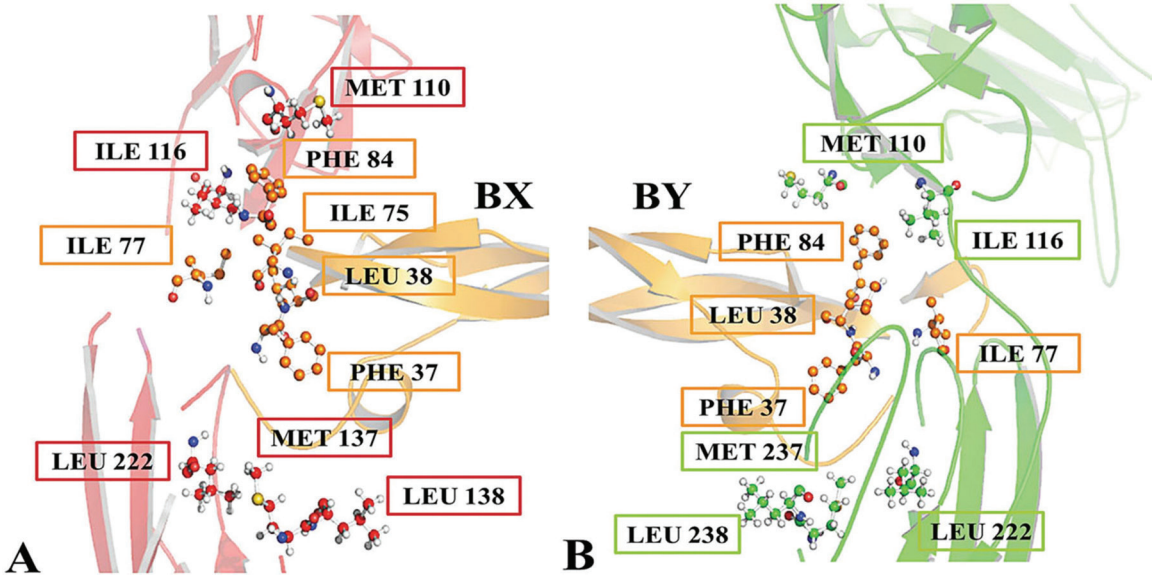

Figure 10. Hydrophobic interface of PDGFB-Ralpha complex were analyzed by DIMLOT: The residues involved in the hydrophobic interactions are depicted as balls and sticks. (a) PDGFBX is in complex with Ralpha monomer (red); (b) PDGFBY binds Ralpha monomer (green). The chain, PDGFAX and PDGFAY do not present a hydrophobic core in their interactions.

Table 1. Contacts between PDGFA and the PDGFRalpha

PDGFA	PDGFRalpha	
PDGFAX	PDGFAY	PDGFAX/PDGFAY
Hydrogen bond		
ARG 159 NH1	ARG 159 N	GLU 298 OE1/ASP 244 OD2
ASN 116 ND2	ASN 116 ND2	ASP 244 OD1/ASN 240 O
	ASN 116 OD1	VAL 243 N
	PRO 112 O	GLU 262 N
	TYR 157 O	THR 296 N
LYS 160 N		ALA 295 O
LYS 161 N		ALA 295 O
SER 114 N		GLU 262 OE1
Hydrophobic interactions		
ALA 115	ALA 115	VAL 243, GLU 262, TYR 273/ GLU 241, VAL 242, TYR 273
ARG 107	ARG 107	ILE 264
ARG 159	ARG 159	ARG 293, ALA 295, THR 296, ARG 297, GLU 298/ ASP 244, ARG 293, ALA 295, LYS 300
ASN 116	ASN 116	GLU 241, VAL 242, ASP 244 ASN 240, GLU 241, VAL 242, TYR 273
ASN 134	ASN 134	GLY 185, PRO 186, ASN 204
ASP 111	ASP 111	MET 260, GLU 262/GLU 262/
GLU 156	GLU 156	VAL 242, THR 296/VAL 242/
LEU 118	LEU 118	TYR 206
LYS 160	LYS 160	ALA 295, THR 296, ARG 297, GLU 298/
		GLN 294, ALA 295, THR 296,

Table 1. Contd...

PDGFA	PDGFRalpha	
		ARG 297, VAL 299
LYS 161	LYS 161	ALA 295/ALA 295, THR 296, GLU 298
PHE 117	PHE 117	GLU 241, VAL 242/GLU 241, VAL 242
PRO 112	PRO 112	THR 259, MET 260, LEU 261, GLU 262/ LEU 261, GLU 262
PRO 162		ILE 139
SER 108		GLU 262
SER 114	SER 114	VAL 243, MET 260, GLU 262, TYR 273/ MET 260, GLU 262, TYR 273
SER 136	SER 136	LYS 270
SER 137	SER 137	VAL 184/VAL 184, LYS 270
THR 113	THR 113	LEU 245, THR 259, MET 260, GLU 262, TYR 273/ THR 259, MET 260, GLU 262
THR 135	THR 135	VAL 184, TYR 206
TYR 157	TYR 157	ILE 139, GLU 141, ALA 295, THR 296/ VAL 242, ASP 244, GLN 294, THR 296, ARG 297
VAL 138		VAL 184
VAL 155	VAL 155	LEU 137, VAL 242/VAL 242
VAL 158	VAL 158	ALA 295, THR 296, GLU 298/ ASP 244, ARG 293, ALA 295, GLU 298
VAL 95		GLU 165
	GLU 90	MET 133
The table discriminates each PDGFA monomers (X and Y)		

Table 2. Contacts between PDGFB and the PDGFRalpha

PDGFB	PDGFRalpha		
PDGFAX	PDGFAY	PDGFAX/PDGFAY	Distance (Å)
Hydrogen bond			
ARG 159 NH1	ARG 159 N	GLU 298 OE1/ASP 244 OD2	2.6.2/2.3.5
ASN 116 ND2	ASN 116 ND2	ASP 244 OD1/ASN 240 O	2.0.9/2.6.8
	ASN 116 OD1	VAL 243 N	2.8.3
	PRO 112 O	GLU 262 N	2.6.4
	TYR 157 O	THR 296 N	3.2.0
LYS 160 N		ALA 295 O	2.5.1
LYS 161 N		ALA 295 O	3.3.0

Contd...

Table 2. Contd...

PDGFB	PDGFRalpha		
SER 114 N		GLU 262 OE1	3.0.5
Hydrophobic interactions			
ALA 35	ALA 35	VAL 242, VAL 243, LEU 245, MET 260, TYR 273/ VAL 243, TYR 273	
ARG 27	ARG 27	GLU 241, VAL 242, VAL 243, GLU 262/ GLU 241, VAL 242, LYS 270	
ARG 28	ARG 28	GLU 262/LEU 261, GLU 263	
ARG 32	ARG 32	THR 259, MET 260, LEU 261, GLU 262/ MET 260, LEU 261, GLU 262	
ARG 56	ARG 56	PRO 267, LYS 270/TYR 206, GLU 241, LYS 270	
ARG 73	ARG 73	MET 133, THR 134/MET 133	
ARG 79	ARG 79	ALA 295, GLU 298/ALA 295	
ASN 34	ASN 34	VAL 243, ASP 244, LEU 245, GLN 246, MET 260, GLU 262, TYR 273	
ASN 36	ASN 36	VAL 242, VAL 243, ASP 244/VAL 243, ASP 244	
ASN 54	ASN 54	VAL 184, GLY 185, PRO 186, ASN 204, VAL 205, TYR 206	
ASN 55	ASN 55	VAL 184, TYR 206, GLU 241/VAL 184, TYR 206	
ASN 57	ASN 57	VAL 184, ALA 207/VAL 184, LYS 209	
ASP 31	ASP 31	GLU 262/GLU 262	
ILE 75		VAL 242	
ILE 77	ILE 77	VAL 242, ASP 244, GLN 294, THR 296/ ILE 139, VAL 242, THR 296	
LEU 38	LEU 38	GLU 241, VAL 242/GLU 241	
LYS 80	LYS 80	GLU 141, ALA 295, THR 296/ILE 139, GLU 141	
PHE 37	PHE 37	VAL 242/VAL 242	
PHE 84	PHE 84	MET 133, THR 134/MET 133, THR 134	
PRO 82	PRO 82	ILE 139/THR 134	
THR 33	THR 33	THR 259, MET 260/THR 259, MET 260, GLU 262, TYR 273	
VAL 58	VAL 58	THR 183, VAL 184/VAL 184	
VAL 78	VAL 78	ALA 295, GLU 298/ALA 295	
	THR 101	THR 183	
The table discriminates each PDGFB monomers (X and Y)			

PDGFA and PDGFB. Additionally, we assessed the 3D structure of Ralpha and reported the possible mechanisms by which Ralpha is more promiscuous and lesser specific than Rbeta. These results may be used as an input for a better understanding of Ralpha/ Rbeta complexes interaction and associated molecular mechanisms. This might be important for drug and peptide design, as elucidating key residues in the interaction of these receptors could increase the specificity of PDGFRs inhibitors.

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por mitógenos en un modelo de toxicidad in vitro; ID 4327 to GB: The ability of GRP78 targeting specific astroglia to protect from traumatic brain injury in vitro and ID 3548 to LM: Efecto de péptidos sintéticos en la función de PDGF.

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Abbreviations: PDGFR, Platelet derived growth factor receptor; PDGF, Platelet derived growth factor; Ralpha, Platelet derived growth factor receptor alpha; Rbeta, Platelet derived growth factor receptor beta; VEGF, Vascular endothelial growth factor; MSA, Multiple sequence alignment; MD, Molecular dynamics; PDGFAX, Platelet derived growth factor A chain X; PDGFAY, Platelet-derived growth factor A chain Y; PDGFBX, Platelet derived growth factor B chain X; PDGFBY, Platelet derived growth factor B chain Y

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