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Molecular modeling of modified peptides, potent inhibitors of the xWNT8 and hWNT8 proteins

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Abstract

Signaling pathways of Wnt-proteins and Fzd-receptors play important role in processes of growth and development of stem cells and in many types of cancers. The binding of the Wnt-proteins and Fzd-receptors is a complicated process, in which 19 Wnt-proteins and 10 Fzd-receptors are involved. Such a large number of combinations of Wnt–Fzd pairs leads to many different influences of Fzd–Wnt-complexes on the development and differentiation of stem cells.

The molecular models of xWnt8, hWnt8, mFzd8, hFzd8-proteins and their complexes were constructed and studied in the present work. The amino acids of the binding sites of proteins which participate in these complexes formation and the protein–protein interactions were studied. The pharmacophoric model of the binding site on the xWnt8 and hWnt8-proteins was constructed. In this work we suggested the peptidomimetic ligands, which can be used for the inhibition of the xWnt8–mFzd8 and hWnt8–hFzd8 proteins formation.

The *de novo* design method of Allegrow software was used for the predictions of most prospective functional groups of the peptidomimetic ligands. These ligands can be used as inhibitors of xWnt8–mFzd8 and hWnt8–hFzd8 complex formation and also can be used for drug design by other methods.

Keywords: Wnt; Frizzled; β-catenin; Cancer; Stem cells

1. Introduction

The signaling pathway Wnt-Frizzled(Fzd)-β-catenin is known to play an important role in the process of regulation of stem cells functioning and also is known to be involved in the carcinogenesis. Inhibition of this signaling pathway is known to downregulate the growth of different tumors [1–3]. Inhibitors of the Wnt–Fzd-signaling pathway represent significant interest as effective anticancer drugs.

Activation of the Fzd-receptors by the Wnt-protein regulates expression of key genes, which are responsible for stem cells differentiation, proliferation, cell polarity and growth [2]. Families of Wnt-proteins and Fzd-receptors are characterized for different vertebrates species. For *Homo Sapiens* 19 members of the Wnt-proteins family and 10 members of the Fzd-proteins family are known. There are also several Fzd-like proteins, which serve as Wnt-inhibitors and Frizzled antagonists [4,5].

Fzd-receptors consist of extracellular, transmembrane and intracellular parts. The extracellular part consists of an N-terminal signaling peptide and a cystein rich domain (CRD-domain) of 120 amino acids, which is linked with the transmembrane part of the receptor [6]. It was shown by point mutagenesis that the CRD-domain is important for the Fzd–Wnt interactions [7]. For activation of the Wnt-Frizzled-β-catenin signaling pathway and for effective Wnt–Fzd interactions, dimerization of CRD domains of Fzd receptors is important [8]. The transmembrane domain of Fzd-receptors contains seven α-helices and six hydrophilic intracellular and extracellular loops. Fzd-receptors also have an intracellular C-terminal site, which is responsible for the signal transduction [9–11].

There are several steps at which the Wnt–Fzd-β-catenin signaling pathway can be inhibited (Fig. 1) [12]. The first step is the binding of a Fzd-receptor with a Wnt-ligand, which is

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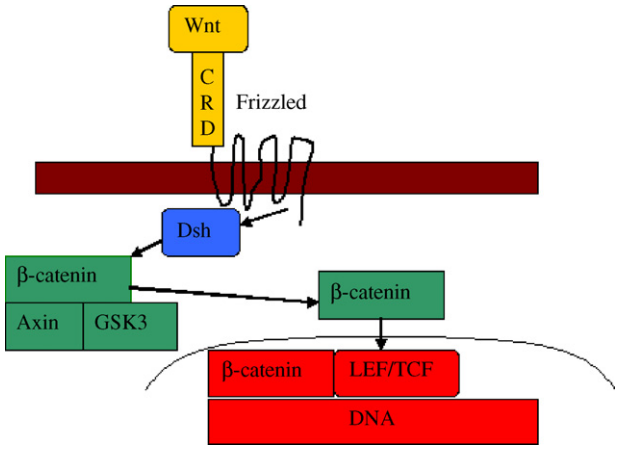

Fig. 1. Main steps of the β-catenin signaling pathways.

regulated by a family of the extracellular Fzd-like proteins [13]. The second step is the interaction of Fzd-receptors with the signaling protein Dsh, which is responsible for the signal transduction into the cell. The third step is the β-catenin ubiquitination in the degradation complex, which contains GSK-kinase, axin, APC and several additional proteins. The fourth step is the formation of a complex between β-catenin and transcription factors LEF/TCF, which regulate activity of many genes responsible for the cell cycle, proliferation and differentiation of stem cells [14].

Because of low solubility of Wnt-proteins the Wnt–Fzd interactions are poorly studied and there is no X-ray structure of the Wnt-proteins [15]. There is also no data available on the spatial structure of the human Fzd-receptors. In this connection modeling of human Wnt and Fzd-proteins becomes an important problem. The aim of this work is to construct computer-aided molecular models of human Wnt-proteins and Fzd-receptors, which may be further used for design of selective inhibitors of Wnt–Frizzled interactions.

Binding of Wnt to Fzd is a poorly understood step in the Wnt–Fzt-β-catenin signaling pathway. The broad diversity of the Wnt-proteins and the Fzd-receptors leads to numerous potential Fzd–Wnt combinations and different influence of these combinations on the differentiation pathways of the stem cells [5]. Selective action on different Fzd–Wnt complexes may lead to selective regulation of stem cells differentiation and functioning.

2. Methods

In this work we describe the construction of molecular models of two Wnt-proteins (xWnt8 and hWnt8), two Fzd-receptors (mFzd8 and hFzd8), their complexes, and peptidomimetics that can be used for the inhibition of such complexes formation. Our primary goal is to construct a human Wnt8 molecular model, a human Frizzled 8 molecular model, and a complex of those two models. The xWnt8–mFzd8 interactions were earlier studied experimentally by point mutagenesis [7]. We set out to use homology modeling for making molecular models of a hWnt8 complex with a CRD-dimer of the human Frizzled8(hFzd8)-receptor. As a template protein for molecular modeling of the hFzd8-receptor CRD-domain, the X-ray structure of mFzd8 CRD-domain (PDB code is 1IJY) was used [7]. As a template for molecular modeling of hWnt8 protein a molecular model of the xWnt8-protein is used. The model of xWnt8 protein and the model of its complex with a CRD-domain of the mFzd8-receptor were previously reported by our group [16]. We used a hypothesis that one Wnt-protein can bind to several CRD domains, so in our molecular model one Wnt protein binds to two CRD domains. Amino acid sequences of the hFzd8-receptor and the hWnt8-protein for homology modeling were taken from PROSITE database http://www.ncbi.nlm.nih.gov/. For each of these amino acid sequences pairwise alignments with the template amino acid sequences shown in Fig. 2 were constructed. ClustalX software along with the Blossum30 matrix is used for the pairwise alignment [17]. The part of the hFzd8 receptor, which corresponds to the CRD-domain of the mFzd8 receptor (according to the pairwise alignment) is used for further molecular modeling. A homology based substitution is performed for non-identical amino acids according to the pairwise alignment of xWnt8–hWnt8 and mFzd–hFzd8 pairs. This substitution and the homology modeling are done by means of Modeller8v1 software [18]. The same software was used for further optimization of the models constructed. The next step is to restore the disulfide bonds, which correspond to those in the template proteins. The structure of the molecular models of hWnt8-proteins and hFzd8-receptors was further optimized using Sybyl 7.1 software [19]. Then the molecular models were placed in a 8 Å solvent box filled with TIP3P water molecules and optimized by Amber 8 sander energy minimization procedure [20]. Quality of the models is checked by the Procheck software (Fig. 3) [21], and also by the module Check Protein of the Sybyl 7.1 software. We used binding sites on the hWnt8 and hFzd8 surfaces localized in our earlier work [16] by xWnt8 and mFzd8 protein–protein docking.

The molecular models of the xWnt8–mFzd8 and hWnt8–hFzd8 complexes enable us to study the hydrophobic interactions, H-bond formation, and the electrostatic interactions between the xWnt8-protein and the dimer of CRD-domain of the mFzd8-receptor as well as between the hWnt8-protein and the dimer of the CRD-domain of the hFzd8-receptor. Analysis of amino acid residues involved in the protein–protein interactions was performed for the CRD domain. Key amino acids of the CRD-domains were determined for mFzd8 and hFzd8, which form the main interactions with the Wnt-protein. These key amino acids were used then for construction of peptide for CRD-domain imitation (further in text – core peptide).

Positions of the core peptide in the binding site were subjected to an additional optimization in our previous work [19] using the Sybyl 7.1 DOCK module. After the core peptide additional docking we performed geometry optimization of the xWnt8 and hWnt8 complexes with the corresponding core peptides. The neighborhood surroundings

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XWNT8 MQNTTLFILATLLIFCPFFTASAWSVNNFLMTGPKAYLTYSASVAVGAQNGIEECKY
WNT8B MFLSKPSVYICLFTCVLQLSHS-WSVNNFLMTGPKAYLIYSSSVAAGAQSGIEECKY

XWNT8 QFAWERWNCPESTLQLATHNGLRSATRETSFVHAISSAGVMYTLTRNCSMGDFDNCG
WNT8B QFAWDRWNCPERALQLSSHGGLRSANRETAFVHAISSAGVMYTLTRNCSLGDFDNCG

XWNT8 CDDSRNGRIGGRGWVWGGCSDNAEFGERISKLFVDGLETGQDARALMNLHNNEAGRL
WNT8B CDDSRNGQLGGQGWLWGGCSDNVGFGEAISKQFVDALETGQDARAAMNLHNNEAGRK

XWNT8 AVKETMKRTCKCHGISGSCSIQTCWLQLAEFRDIGNHLKIKHDQALKLEMDKRKMRS
WNT8B AVKGTMKRTCKCHGVSGSCTTQTCWLQLPEFREVGAHLKEKYHAALKVDLLQG---A

XWNT8 GNSADNRGAIADAFSSVAGSELIFLEDSPDYCLK-NISLGLQGTEGRECLQSGKNLS
WNT8B GNSAAARGAIADTFRSISTRELVHLEDSPDYCLE—NKTLGLLGTEGRECLRRGRALG

XWNT8 QWERRSCRRLCTDCGLRVEEKKTEIISSCNCKFHWCCTVKCEQCKQVVIKHFCARRE
WNT8B RWELRSCRRLCGDCGLAVEERRAETVSSCNCKFHWCCAVRCEQCRRRVTKYFCSRAE

XWNT8 RDSNMLNTKRKNRGHRR
WNT8B RPRGGAAHKP---GRKP

mFzd8 ELACQEITVPLCKGIGYEYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSM
hFzd8 ELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSM

mFzd8 YTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDY
hFzd8 YTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDY

mFzd8 ER
hFzd8 NR
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Fig. 2. Amino acid sequences pairwise alignment for mFzd8–hFzd8 and xWnt8–hWnt8 pairs.

of each core peptide in the binding site were characterized and used for the prediction of promising modifications of core peptide.

Generation of the core peptides modifications was performed by a *de novo* drug design method within the Allegrow software, which uses the method of fragment libraries [22]. Using this method and based on analysis of the resulting modifications we suggested the most prospective modifications of the core peptides for enhancing their binding affinity to xWnt8 or hWnt8.

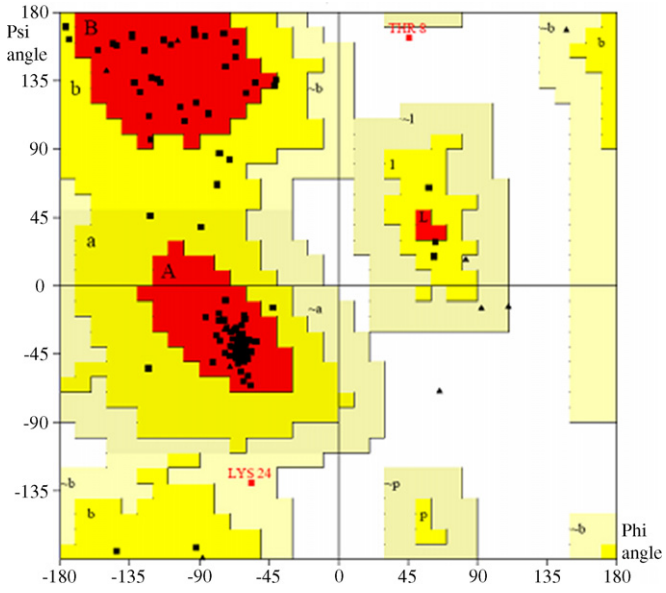

Fig. 3. Quality check of hWnt8-protein model structure. Red field – most favored region [A, B, L], yellow field-additional allowed region[a, b, l, p], light yellow-generously allowed [∼a, ∼b, ∼l, ∼p].

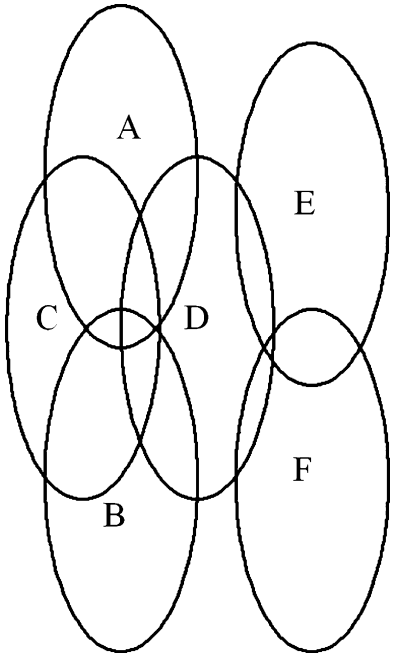

Fig. 4. hWnt8–xWnt8 binding site clusters.

3. Results and discussion

3.1. Binding sites on xWnt8 and hWnt8 surfaces

The molecular models of protein–protein complexes are used for studying the xWnt8 and hWnt8 binding sites amino acids at the distance of 5 Å from amino acids of the mFzd8 and hFzd8 CRD domains (Fig. 4). Based on the binding surfaces we suggest a scheme of the xWnt8 and hWnt8 proteins binding sites. The side chains of amino acids forming those binding sites were studied and a pharmacophore model was constructed for possible small molecule inhibitors of the mFzd8–xWnt8 and hFzd8–hWnt8 complexes.

The xWnt8 and hWnt8 proteins are homological proteins (amino acid sequence identity is 78%). For the CRD-domains the homology is very high in the mFzd8 and hFzd8 receptors, with only two amino acids different (Fig. 2). The numberings of the mFzd8 and hFzd8 amino acids are the same hereinafter.

We have compared the hWnt8-protein molecular model with that of the xWnt8-protein and analyzed differences in their binding sites (Fig. 4). The binding sites of both the xWnt8 and hWnt8-proteins models with a CRD-domain consist of seven different amino acid clusters (Fig. 4), which are labeled A(63–64), B(180–193), C(320–331), D(121–125), E(300–310), F(225–240), with the numbers corresponding to the numbering of the amino acids. These clusters can be classified into two groups (each interacting with one of the two CRD domains of the Fzd receptors): A, B, C and D, E, F, where the D amino acid cluster is located between the A, B, C and the E, F-clusters.

The structure of the xWnt8 and hWnt8 binding sites is shown in Fig. 5. In Fig. 6 the amino acids are placed in the order corresponding to 2D projections of the 3D binding site. The number and the name of xWnt8 amino acids are shown. The corresponding numbers of hWnt8 amino acids are shown in the parentheses and in a case if they are different from xWnt8 their names are also given. Analysis of these amino acids side chains can be used for generating pharmacophore hypotheses for potential inhibitors.

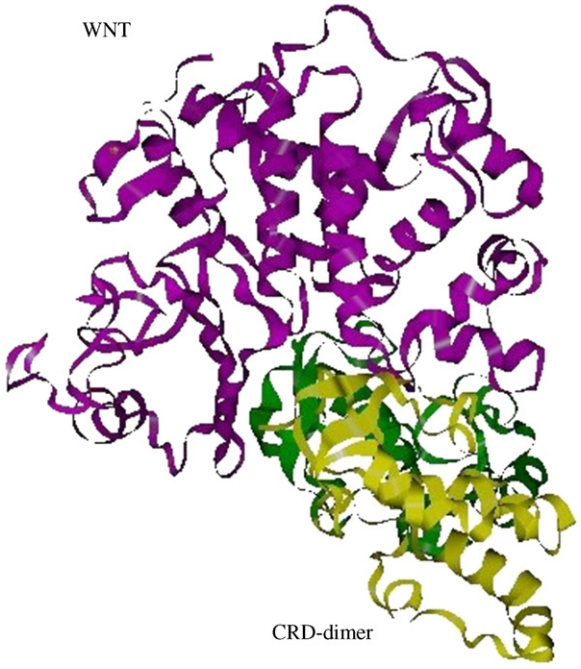

Fig. 5. Structure of hWnt8 complex with dimeric CRD-domain of hFzd8-receptor.

The region around the Gln193(192) amino acid is favorable for H-bond formation with H-bond acceptors. In the case of peptides Glu or Asp can be used, while in the case of non-peptide small molecules they can be carboxyl or carbonyl groups, hydroxyl or amide groups. In the region around Ser191(190), Ser187(186) and Ser189(188) (serine pocket) the most favorable are the hydroxyl, carbonyl and carboxyl groups, amide or amino groups, which can form H-bonds with Ser as a donor and as an acceptor. For peptide ligands the most favorable for this region are Ser and Thr amino acids. For this region the introduction into ligand of amide groups is prospective since the oxygen atom of the amide group interacts with the hydrogen atom of Ser, while the hydrogen atoms of the amide group interact with the oxygen atom of Ser.

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The amino acid residue Arg122 in the xWnt8-protein corresponds to the Lys121 in hWnt8. Both amino acid residues can participate in the bond formation with negatively charged groups, but Arg122 can also participate in the formation of H-bonds with oxygen-containing functional groups of putative ligands. In the xWnt8-protein Ile123 forms a hydrophobic pocket, which can interact with ligand's small size alkyl substituents. In the hWnt8-protein this position corresponds to the Thr122 residue, which can participate both in hydrophobic interactions and in hydrogen bonding. Substituents that fit the best to Thr122 would be hydroxyl or other oxygen-containing groups. The Ile192 amino acid of the xWnt8-protein corresponds to Ile191 in the binding site of the hWnt8-protein. Optimal substituents matching this amino acid would be small hydrophobic substituents, consisting of 1–3 non-hydrogen atoms.

The region between Asp233 and Asn234 is favorable for different types of bonding. The most promising substituents in the ligand for this region would be amide and hydroxyl groups, which can interact both with the amide group of Asn234 and with the carboxyl group of Asp233. The introduction of amino groups is prospective for bonding with Asp233. In the hWnt8

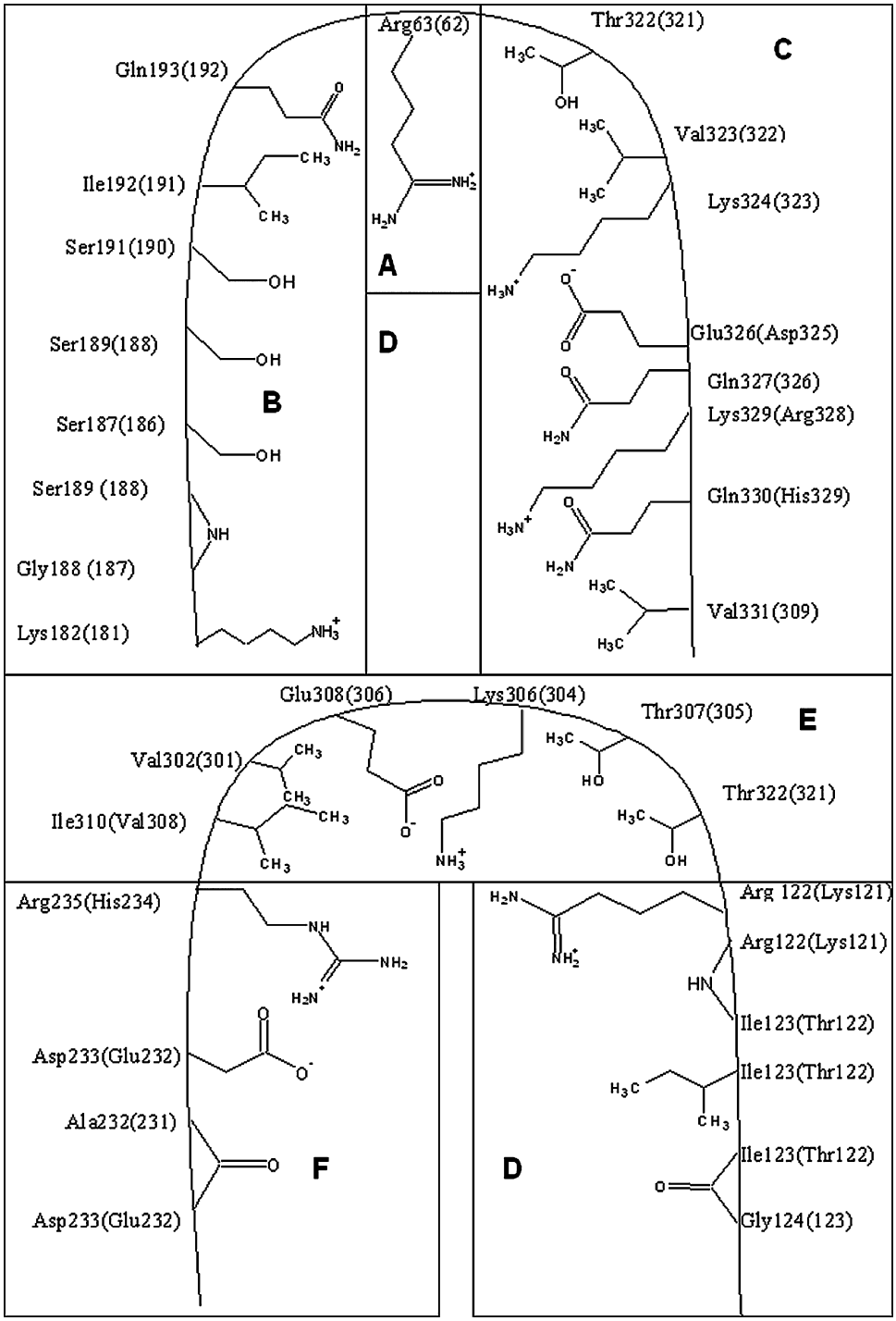

Fig. 6. The amino acids in the hWnt8 and xWnt8 binding site. A, B, C, D, E, F are labels for amino acid clusters.

protein Glu232 corresponds to Asp233 of the xWnt8 protein. The Glu232 amino acid residue is more bulky and can interact with the same amino acids as Asp233, but only with less bulky functional groups or functional groups placed aside of Gly233 can be used.

The amino acid residue Arg235 corresponds to the His234 residue, which can form bonds with negatively charged functional groups, but in the case of the hWnt8 protein such functional groups should be longer by several CH_2 units than in the case of the xWnt8 protein. The introduction of hydrophobic 1–4 atomic substituents into ligands is effective for the interactions with the Ile310 amino acid of xWnt8 protein. In the case of the hWnt8-protein the amino acid Val308 corresponds to the Ile310 amino acid of the xWnt8. In the case of hWnt8 protein the hydrophobic region around the Val308 is larger than that of Ile310 and enables one to introduce a hydrophobic substituent with a longer chain. The amino acid residue Glu308(306) can form hydrogen bonds with amino groups and

with hydroxyl and amide groups as well. The amino acids Lys306(304), Lys324(323), Lys329(Arg328) (Lys pocket) potentially can bind many negatively charged functional groups, such as carboxyl or phenolate. In the case of peptide ligands they could be ionized carboxyl groups of the amino acids Glu and Asp. The amino acid residue Arg328 of the hWnt8-protein can also participate in the H-bond formation with the oxygen-containing functional groups. The residue Thr322(321) is promising for binding with hydroxyl-containing fragments and ether groups. The corresponding peptide ligands can contain Ser or Thr amino acids. The amino acid Val323(322) favors hydrophobic groups of the ligand such as Me, Et, *n*-Pr, or alkoxy groups such as OMe and OEt. The Glu326 and Gln327(326) site can interact with functional

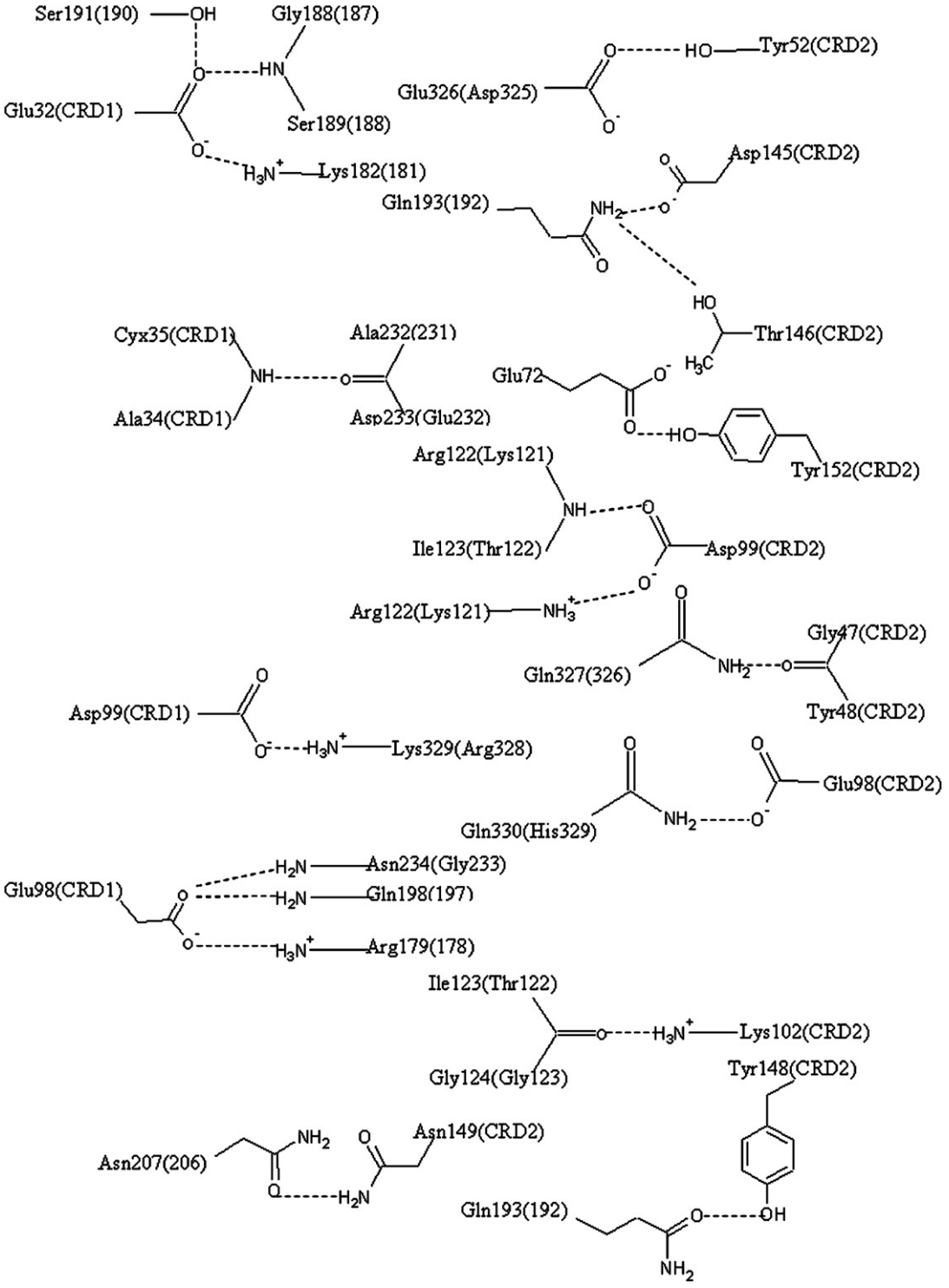

Fig. 7. The interactions mFzd8–xWnt8 and hFzd8–hWnt8. CRD1 and CRD2 show corresponding domains of Fzd-receptors.

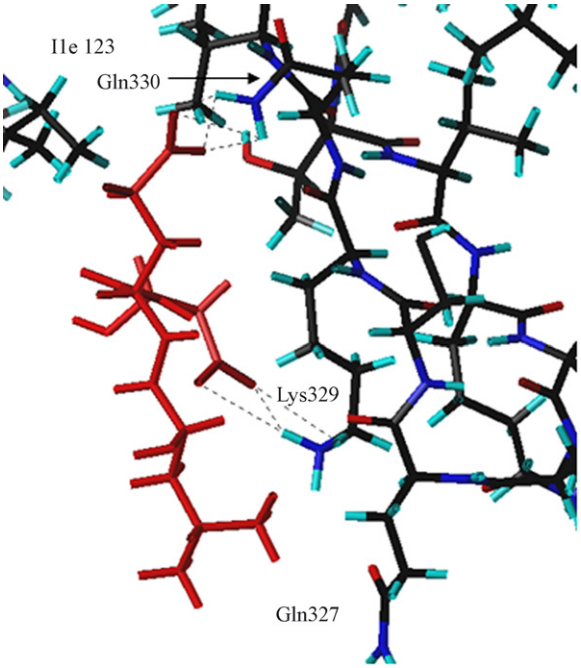

Fig. 8. The example of peptidomimetic in the binding site on the hWnt8-protein surface.

groups having H-bond donors and H-bond acceptors. Amide and hydroxyl groups are promising for the interaction with this region as far as such groups can interact both with Glu and Gln. The Glu326 in the binding site of the xWnt8-protein corresponds to the Asp325 in the binding site of the hWnt8-protein. For this region in the case of the hWnt8-protein the same functional groups can be introduced into the ligand as well as in the case of xWnt8 protein, but for the hWnt8 protein bulkier functional groups can be used. The neighborhood of the xWnt8 protein's Gln330 favors the carbonyl and carboxyl groups, which can interact with amino and hydroxyl groups. The Gln330 of the xWnt8 protein corresponds to the His329 in the hWnt8 protein, which may be interesting since the Glu330 is negatively charged, and His329 is charged positively. This is the most important difference between the two binding sites. The His329 as well as the Gln330 can form an H-bond with the oxygen-containing functional groups and also can interact electrostatically with negatively charged groups such as carboxyl and phenolate. In Fig. 7 a scheme of interactions of the dimeric CRD-domain amino acids of the mFzd8 and hFzd8 receptors with the xWnt8-protein and hWnt8-protein is shown. The H-bonds and electrostatic interactions are shown in Fig. 7, with different CRD subunits labeled as CRD1 and CRD2.

3.2. Interactions in the complexes mFzd8–xWnt8 and hFzd8–hWnt8

The monomeric xWnt8-protein interacts with each of the two CRD-domains of the mFzd8-receptor. The amino acid residue Glu32 of the CRD1-domain carboxyl group forms a hydrogen bond with the Ser191(190) hydroxyl group and also interacts electrostatically with the Lys182(181). It also forms an H-bond through the NH-group of Ser189(188). The NH-group of the Ala34 amide residue forms an H-bond with the Ala232(231) carbonyl group. The Asp99 of the CRD1-domain interacts electrostatically with the Lys329(Arg328). The carboxy group of the Glu98 of the CRD1-domain interacts with the Arg179(178) and forms H-bonds with the Asn234(Gly233) and Gln198(197). The Tyr52 hydroxyl group of the CRD2 domain forms an H-bond with the carboxyl group of the Glu326(Asp325). The Asp145 carboxyl group of the CRD2 domain forms a hydrogen bond with the amide group of the Gln193(192). The amide group of the Gln193(192) forms hydrogen bonding with the oxygen of the hydroxyl group of the Thr146 of the CRD2-domain. The Asp99 of the CRD2-domain interacts electrostatically with the Arg122(Lys121) and also forms a hydrogen bond with the NH-group of the Ile123(Thr122). The CO group of the Tyr48(CRD2) forms an H-bond with the amide group of the Gln327(326). The amide group of the Gln330(His329) forms an H-bond with the carboxyl group of the Glu98(CRD2).

According to the model proposed, the amino acids of sites 30–35, 48–52 and 140–146 of the CRD-domain of the mFzd8 and hFzd8-receptors are important for the binding with hWnt8 and xWnt8. These amino acids correspond to the fragments which were previously shown experimentally [7] to be important for the binding of the xWnt8-protein that provides an additional proof of the adequacy of the model.

These protein–protein interactions were predicted by us on the basis of the models constructed and can be used for directed point mutagenesis of the corresponding amino acids of xWnt8 and hWnt8 proteins. These point mutations can be used in turn for the evaluation of quality of the xWnt8 and hWnt8 models and their complexes with the mFzd8 and hFzd8 receptors.

3.3. Design of modified peptides as promising inhibitors

As far as Wnt-proteins or Frizzled-receptors have no small molecules which can inhibit their interactions, the first step of development of such inhibitors is the design of modified peptides. The example of such modified peptide is shown in Fig. 8. Affinity of peptide inhibitors towards the target protein can be enhanced by additional modifications of these peptides shown in Fig. 9, which would allow formation of additional bonds with the target proteins. The core peptide which mimics the CRD-domain is shown in Fig. 9 (ligand 9) in which Rn is the position of the modification suggested. The structures of the corresponding Rn are also shown.

The introduction of the R1-substituent shown in the ligand 1 results in additional H-bonds with the NH_2-group of the amino acid Arg122 in the xWnt8-protein. The Arg122(xWnt8) corresponds to the Lys121 amino acid in the hWnt8-protein. Both the amino acids are likely to participate in the electrostatic interactions.

The ligand 2 has a hydroxyl group in the R1-substituent, which can lead to the H-bond formation with the

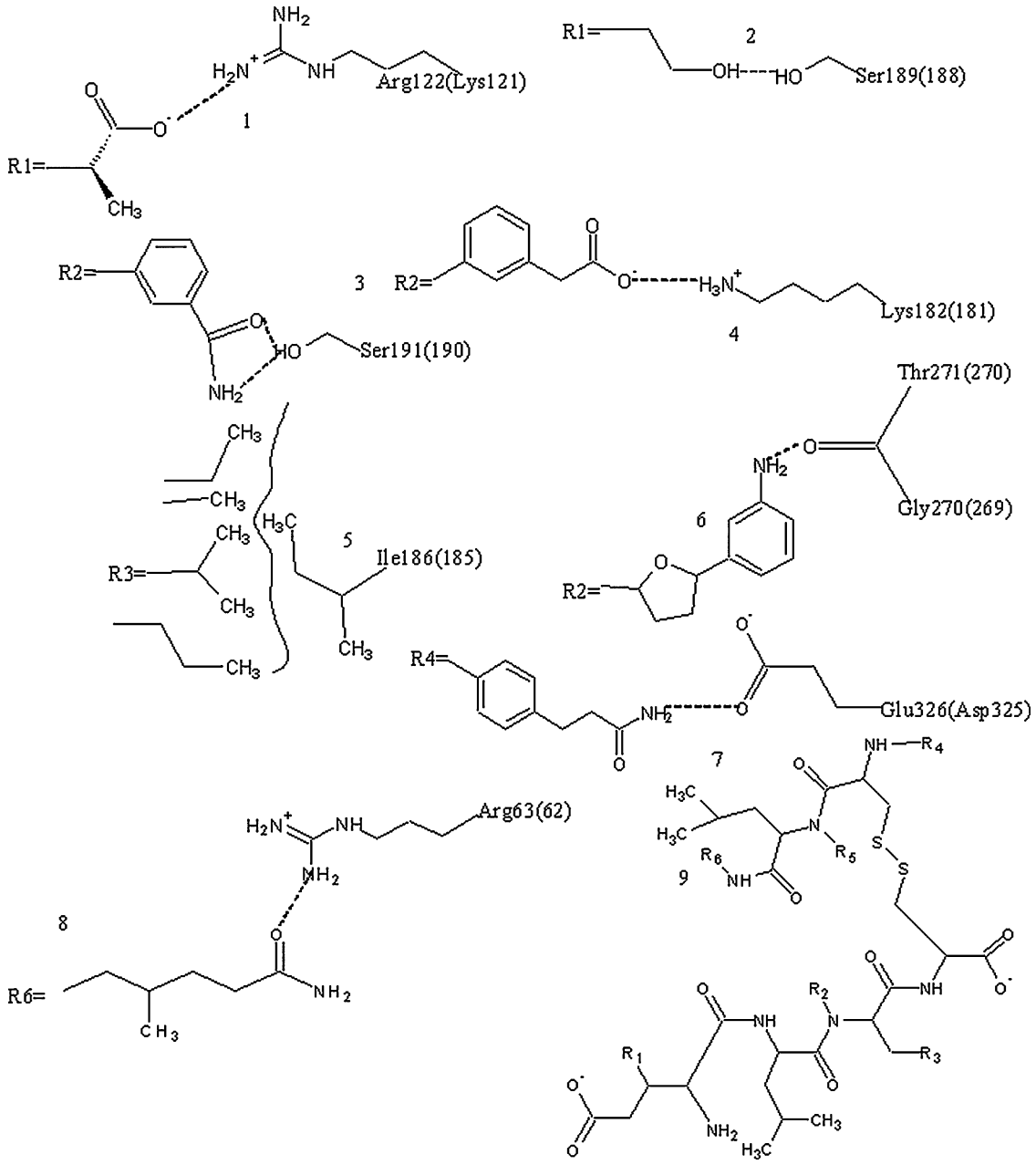

Fig. 9. Examples of the modified peptides as potential inhibitors of the Wnt-Frizzled signaling pathway. Numbers correspond to ligand numbering, and Rn is the numbering of R in the core peptide; 9 – core peptide.

Ser189(188). For the ligand 3 the functional groups which can form H-bonds with the hydroxyl group of Ser191(190) are favored. For designing ligand 4 we exploited an opportunity of electrostatic interactions of the ligand functional group with the positively charged Lys182(181). In the group of ligands 5 the small hydrophobic substituents enhance hydrophobic interactions with the protein. In the ligand 6 the substituent participates in the H-bond formation with the carbonyl group of the Thr271(270). In the ligand 7 the possibility of hydrogen bonding with Glu326 is used. For the region of the Asp325 of the hWnt8-protein the respective carbon chain of the ligand 7 should be longer. In the ligand 8 the substituent forms an H-bond with the NH_2-group of the Arg63(62).

4. Conclusions

In this work we constructed and analyzed molecular models of the complexes of the xWnt8-protein with the CRD-domain of the Fzd8-receptor as well as the hWnt8-protein with the CRD-domain of the hFzd8-receptor. The binding sites on the surface of the xWnt8 and hWnt8-proteins and on the surface of CRD-domains of the mFzd8 and hFzd8-receptors are characterized. The differences between the binding sites of the xWnt8, hWnt8-proteins and mFzd8, hFzd8 are analyzed. Possible pharmacophores for potential inhibitors of these complexes are predicted. Modified peptides having enhanced affinity towards the xWnt8 and hWnt8-proteins are suggested. These ligands can potentially be used as the inhibitors of the

formation of the xWnt8–mFzd8 and hWnt8–hFzd8 and can be used for stem cells functioning regulation, and as anticancer agents. Our models and ligands predicted can be used for experimental tests by other researchers.

References

[1] X. He, A Wnt–Wnt situation, Dev. Cell 4 (2003) 791–797.

[2] R. Nusse, Wnt signaling in disease and in development, Cell Res. 15 (2005) 28–32.

[3] E. Vincan, P.K. Darcy, M.J. Smyth, E.W. Thompson, R.J.S. Thomas, W.A. Phillips, R.G. Ramsay, Frizzled-7 receptor ectodomain expression in a colon cancer cell line induces morphological change and attenuates tumor growth, Differentiation 73 (2005) 142–153.

[4] J.R. Miller, The Wnts, Genome. Biol. 3 (2002) 1–15.

[5] P. Bhanot, M. Brink, C.H. Samos, J.C. Hsieh, Y. Wang, J.P. Macke, D. Andrew, J. Nathans, R. Nusse, A new member of the frizzled family from Drosophila functions as a Wingless receptor, Nature 382 (1996) 225–230.

[6] J.M. Chong, A. Uren, J.S. Rubin, D.W. Speicher, Disulfide bond assignments of secreted Frizzled-related protein-1 provide insights about Frizzled homology and netrin modules, J. Biol. Chem. 277 (2002) 5134–5144.

[7] C.E. Dann, J.C. Hsieh, A. Rattner, D. Sharma, J. Nathans, D.J. Leahy, Insights into Wnt binding and signaling from the structures of two Frizzled cysteine-rich domains, Nature 412 (2001) 86–90.

[8] C. Carron, A. Pascal, J.C. Djiane, J.C. Boucaut, D.L. Shi, M. Umbhauer, Frizzled receptor dimerization is sufficient to activate the Wnt/β-catenin pathway, J. Cell Sci. 116 (2003) 2541–2550.

[9] M. Umbhauer, A. Djiane, C. Goisset, A. Penzo-Mendez, J.F. Riou, J.C. Boucaut, D.L. Shi, The C-terminal cytoplasmic Lys-thr-X-X-X-Trp motif in frizzled receptors mediates Wnt/β-catenin signaling, EMBO J. 19 (2000) 4944–4954.

[10] G. Liu, A. Bafico, S.A. Aaronson, The mechanism of endogenous receptor activation functionally distinguishes prototype canonical and noncanonical Wnts, Mol. Cell. Biol. 25 (2005) 3475–3482.

[11] J. Wu, T.J. Klein, M. Mlodzik, Subcellular localization of frizzled receptors, mediated by their cytoplasmic tails, regulates signaling pathway specificity, PLoS Biol. 2 (2004) 1004–1014.

[12] N. Janssens, M. Janicot, T. Perera, The Wnt-dependent signaling pathways as target in oncology drug discovery, Investig. New Drugs 24 (2006) 263–280.

[13] Y. Kawano, R. Kypta, Secreted antagonists of the Wnt signalling pathway, J. Cell Sci. 116 (2003) 2627–2634.

[14] D.D. Armstrong, K.A. Esser, Wnt/β-catenin signaling activates growth-control genes during overload-induced skeletal muscle hypertrophy, Am. J. Physiol. Cell Physiol. 289 (2005) 2–31.

[15] J. Hsieh, A. Rattner, P. Smallwood, J. Nathans, Biochemical characterization of Wnt-Frizzled interactions using a soluble, bioactive vertebrate Wnt protein, Proc. Natl. Acad. Sci. 96 (1999) 3546–3551.

[16] A.E. Voronkov, I.I. Baskin, V.A. Palyulin, N.S. Zefirov, Molecular modeling of the complex between the xWNT8 protein and the CRD domain of the mFZD8 receptor, Dokl. Biochem. Biophys. 412 (2007) 262–267.

[17] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucl. Acids Res. 25 (1997) 4876–4882.

[18] A. Sali, T. Blundell, From protein comparison of protein sequences and structures to protein modeling and design, Mol. Biol. 234 (1993) 779–815.

[19] SYBYL 7.1, Tripos Inc., 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.

[20] D.A. Case, T.E. Cheatham, T. Darden, The Amber biomolecular simulation programs, J. Comput. Chem. 26 (2005) 1668–1688.

[21] R. Laskowski, M. MacArthur, D. Moss, *PROCHECK*: a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283–291.

[22] C. McMartin, R.J. Bohacek, QXP: Powerful, rapid computer algorithms for structure-based drug design, Comput-Aided Mol. Des. 11 (1997) 333–344.