A Thesis for Master of Science

In Silico screening for potential Developmentally Regulated GTP Binding Protein 2-interacting metabolites

The Graduate School

of the University of Ulsan

Department of Biological Science

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In Silico screening for potential Developmentally Regulated GTP Binding Protein 2-interacting metabolites

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ABSTRACT

Developmentally regulated GTP-binding protein 2 (DRG2) is involved in various physiological functions. There are currently no reports on the role of DRG2 in cellular metabolisms. To get insights into the role of DRG2 in metabolism, we analyzed the interactions between DRG2, and 420 metabolites collected from the Human Metabolome Database (HMDB) by computational docking studies. In silico molecular docking analysis identified 37 metabolites showing binding energy with DRG2 higher than GTP (-6.9 kj\mole). Most of them were involved in steroid hormone metabolism. We also determined the amino acid residues of DRG2 involved in the interaction with these metabolites. We demonstrated that some metabolites share amino acid residues of DRG2 for their binding with GTP, suggesting that these metabolites may compete with GTP for binding with DRG2 and thus affect DRG2 activity. BioMuta (a database of cancer-associated single-nucleotide variations) analysis revealed variations within the amino acid residues of DRG2 responsible for binding with these metabolites in human cancer cells. These suggest that DRG2 may interact with hormones and play important roles in the regulation of hormone metabolism and that disruption of these interactions may affect the growth and metastasis of cancer cells.

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USEFUL ABBREVIATION AND TERMINOLOGY

AMP: Adenosine monophosphate.

ATP: Adenosine triphosphate.

DRG2: Developmentally regulated GTP-binding protein.

GTP: Guanosine triphosphate.

GDP: Guanosine diphosphate.

nsSNV: Non-synonymous single nucleotide variations.

NCBI database: Database of National Center for Biotechnology Information.

A°: Angstrom. Metric unit of length equal to 10^{-10} m.

Homology modelling: Protein homology modelling refers to constructing an atomic-resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein. Evolutionarily related proteins have similar sequences and naturally occurring homologous proteins have similar protein structure.

Virtual Screening: Virtual screening is a computational technique used in drug discovery to search libraries of small molecules in order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme. It provides an inexpensive and fast alternative to high-throughput screening for discovering new drugs or interacting metabolites.

Molecular docking: The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the

behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes.

Variant: Variant is a member of a set of highly similar proteins that originate from a single gene or gene family and are the result of genetic differences. While many perform the same or similar biological roles, some isoforms have unique functions. A set of protein isoforms may be formed from alternative splicing, variable promoter usage, or other post-transcriptional modifications of a single gene. DRG2 A124T is a variant of DRG2 protein where it describes that the 124^{th} no. alanine (A) residue in DRG2 protein sequence is changed to Threonine (T).

Model Validation: It is a step after constructing a homology model where the model is examined for accurate of each atom's location. To find accurate protein-metabolite interaction it is necessary to construct a very reliable protein model. The validation step approves the model for further interaction investigation. Rampage server is widely used Validation software to provide information of model validity.

Binding affinity: when a metabolite binds or interacts with a protein, it releases some energy. It's called binding energy. The strength of the binding is the binding affinity of the metabolite. It was showed that, lesser binding energy have more binding affinity of the metabolite against the protein model.

Binding site: Binding site is a region in a protein 3D model where a specific metabolite binds or interacts. A protein model has several binding sites for a metabolite. The site which shows least binding energy was selected as the most accurate binding site for that metabolite.

1. INTRODUCTION

Natural small metabolites interact with various protein enzymatically as substrates or products, or allosterically as cofactors or ligands. By using metabolite-interacting proteins, cells can percept the dynamic changes in intracellular and extracellular metabolites and respond to the changing environment by modulating cell signaling and gene expression [1-3]. A well-known example is the lactose-lac repressor binding. The binding of lactose prevents the lac repressor from binding to DNA [4]. In the case of bacterial pyruvate kinase, binding of AMP, ribose-5-phosphate and glucose-6-phosphate activate the kinase activity, while binding of ATP and ortho-phosphate inhibits the kinase activity [5]. Thus, networks of metabolites interacting with a protein can provide valuable information about signaling and necessary biochemical pathways modulated by that protein [6-8].

Recently, with the advances in computer science, various in silico methods have been developed for the analysis of metabolite-protein interactions, using different software tools [9]. These tools can use different computational techniques to examine the binding energy, binding sites, and docking between metabolites and proteins. If the structure of the binding site of a target protein is known, accurate protein structure in complex with small molecules can be reliably predicted by using docking tool [10-13]. If the three-dimensional structure of the target protein is unavailable, a homology model can be created from its amino acid sequence and the available structures of its template homologous proteins [14].

Guanine triphosphate (GTP) is one of the major cellular metabolites and served as a building block for RNA and DNA as well as an energy source to drive cellular activities such as intracellular trafficking, the cell migration and translation. G proteins, also known as GTP-binding protein, are

family of proteins that can efficiently bind and subsequently hydrolyze GTP. They are involved in transmitting signals from a variety of stimuli outside a cell to its interior and their activity depends on the binding of GTP or GDP: when they are bound to GTP, they are active, and when they are bound to GDP, they are inactive. Developmentally regulated GTP-binding proteins (DRGs) are a novel class of GTP-binding protein [15] consisting of two closely related proteins, DRG1 and DRG2 [15, 16]. The evolutionary conservation of DRGs suggests that they may play an essential role in the control of cell growth and differentiation. DRG1 and DRG2 interact with different molecules, DFRP1 and DFRP2, respectively [17], suggesting that they have distinct functions. Overexpression or knockdown of human DRG2 causes cell cycle arrest [18-20]. In addition, DRG2 overexpression inhibits NF-κB function and interleukin-6 production in macrophages, as well as TH17 differentiation, and ameliorates experimental autoimmune encephalomyelitis in mice [21-27]. Interestingly, knocking out DRG2 leads to defect in striatal dopamine release and impairs the functions of dopaminergic neuron in mice [28]. Recently, it has been shown that DRG2 knockdown decreases the stability of Rac1-positive membrane tubules [29-32], induces mitochondrial dysfunction [33], and decrease the growth of primary melanoma and lung metastasis by inhibiting VEGF-A production [34]. All these reports suggest that DRG2 plays important roles in the regulation of cell signaling, energy metabolism, cell growth, and cell migration. However, despite their essential roles in biological processes, the precise mechanism of how DRG2 senses the changes in the metabolites and responds to the changing environment is still mostly unknown.

Until now, there have been no reports on the DRG2-interacting metabolites except the GTP. In this study, we determined whether DRG2 interacts with metabolites using a silico method. To determine whether DRG2 can sense the changes of metabolites in the extracellular and cytoplasmic environments, we selected 420 metabolites which are found in cytoplasm and blood from Human

Metabolome Database (HMDB) which is a freely available electronic database storing 114,482 metabolites found in human body [35,36]. In silico analysis of DRG2-metabolites binding revealed 37 metabolites with binding affinity higher than GTP which are enriched in hormone metabolisms. We also demonstrated that some metabolites share amino acid residues of DRG2 for their binding with GTP, suggesting that these metabolites may compete with GTP for binding with DRG2 and thus affect DRG2 activity.

2. MATERIALS AND METHODS

2.1 Generation and Validation of DRG2 3D Homology model

The primary amino acid sequence of DRG2 protein was collected from NCBI database (NCBI reference sequence NP_001379.1, GI: 4557537). PHYRE2 server was used for constructing the homology model for DRG2 according to that amino acid sequence [37]. Rampage server was used for validation of the 3d homology model constructed by PHYRE2 sever [38].

2.2 Molecular docking and Virtual screening of DRG2

Autodock Vina was used for docking analysis of DRG2 protein [39]. Metabolites from Human Metabolome Database (HMDB: http://www.hmdb.ca/metabolites) database, which are detected and quantified earlier, found in blood, endogenous, and locates in the cytoplasm are selected for docking. Based on these 420 metabolites were filtered from HMDB and selected as ligands for docking analysis with DRG2. The grid box was set to 82A° x 82A° x 102A° with 1.0A° with spacing between the grid points for covering the whole protein in order to remove any bias. 9 conformers were generated for each metabolite. First conformer which showed the least binding energy was selected for further analysis. Pymol program was used for visualizing the conformer and protein interaction [40]

2.3 nsSNVs of DRG2

The Biomuta server listed all nsSNVs of DRG2 in various human cancer cells (https://hive.biochemistry.gwu.edu/biomuta/proteinview /P55039). We downloaded the table of all unique variation of DRG2 with their altered residue, frequency, and cancer types.

2.4 Generation of 3D homology model of DRG2 A124T, A124V and S165F variants

DRG2 A124T, DRG2 A124V and DRG2 S165F variants 3D homology model was constructed by the help of Phyre2 server. For obtaining DRG2 A124T, A124V and S165F variants amino acid sequences, the residue was changed in DRG2 amino acid sequence collected from NCBI database (NCBI reference sequence NP_001379.1, GI: 4557537) respectively. For visualization of the variants, Pymol program was used. Rampage server was used for homology 3D model validation.

2.5 Molecular docking and Virtual screening of DRG2 A124T, A124V and S165F variants

Docking analysis for these three variants was served by Autodock Vina program. The grid box was set to 84.764A° x 31.804A° x 26.360A° with 1.0A° spacing for DRG2 A124T and DRG2 A124V and DRG2 S165F variants for covering the whole protein model. The Top 37 metabolites of DRG2 was selected as ligands for docking analysis for the variants. Phymol program is used for later screening the docking between each metabolite with each variant.

2.6 Identification of binding residues of DRG2 and its variants

Phymol program is used for identifying the binding residues for each metabolite against DRG2 and each of its variants. Visualization was also done by Phymol program.

3. RESULTS

3.1 Generation and Validation of 3D Homology model of DRG2 protein

Until now, the three-dimensional structure of DRG2 has not been determined. Thus, a homology 3D model of DRG2 was constructed using Phyre2 server based on 364 amino acid sequence collected from NCBI (Fig. 1). Rampage server validated the stereo-chemical properties of this 3D homology model. The stereo-chemical quality data of DRG2 3D model obtained from Rampage server shows that 327 residues out of 364 which cover 90.3% amino acids of DRG2 are in the favored region. 24 residues (6.6%) are in the allowed region and 11 residues (3%) are in the outlier region (Fig. 2). The overall accepted region of DRG2 3D model is 96.9%, which is suitable for good quality protein model.

3.2 Molecular docking and virtual screening for DRG2-interacting metabolites

We selected 420 metabolites which are found in cytoplasm and blood from the Human Metabolome Database (HMDB) [41, 42]. The 420 metabolites were docked against DRG2 protein, where the grid volume was covered the whole area as the binding site is unknown. The nine conformers for each metabolite were generated, and the conformers having the highest binding affinities were selected. The binding energies of 420 metabolites were ranged from -1.4 kj/mol to -10.2 kj/mol. Among them, 37 metabolites were found to have binding energy higher than GTP (-6.9 kj/mol) and most of the 37 metabolites were involved in the steroid hormone metabolisms (Table 1). These results suggest that DRG2 may play a role in steroid hormone-metabolism.

3.3 Binding site Detection for DRG2

Pymol program was used to screen the binding sites for the top 11 metabolites in DRG2 protein. Most of them showed similar binding regions in DRG2 protein (Fig. 3). The regions of DRG2 involved in binding with these metabolites were shown in Figure 3 and the amino acid residues in the binding sites of DRG2 were listed (Table 2).

3.4 Identification of SNVs in DRG2

By using BioMuta analysis, we selected SNVs which were found in the human cancer within DRG2 coding region and induced amino acid substitution. A total of 83 SNVs were identified to induce amino acid substitution within DRG2 protein. Among them, 5 SNVs were found to induce amino acid substitutions in binding sites of DRG2 with top 9 metabolites (Table 3).

3.5 Generation and Validation of 3D Homology model of DRG2 A124T, A124V and S165F Variant

3D homology model of DRG2 variants were constructed using Phyre2 server based on 364 amino acid sequence collected from NCBI. The altered residue was changed in the sequence for each variant. Pymol program was used for visualization (Fig. 4). From Rampage server it was found that the residues in favored region were 94.5%, 93.1% and 94.5% for DRG2 A124T, A124V and S165F variant respectively. It assured that the protein 3D structures of the variants were validated for further docking analysis.

3.6 Molecular docking of Top 37 metabolites against the DRG2 Variants

The top 37 metabolites were docked against DRG2 A124T, A124V and S165F variant. The binding energy was found changed for each metabolite with each variant. But most metabolites showed very little change against the variant (Table 4).

3.7 Binding site Detection for DRG2 Variants

The binding sites for top 11 metabolites, GTP and GDP were identified by Pymol program for DRG2 A124T, A124V and S165F variants (Fig 5-7). The binding regions are found to be changed for variants compare with DRG2. The residues involved in the binding regions showed dramatically changed compare with DRG2 (Table 5). As the binding energies were not changed so much but binding residues were found drastically changed it is assumed that, for achieving least or similar binding affinity the metabolites changed the binding regions in the variants. The changes in the variants of DRG2 let the metabolites change their binding regions as well as binding residues.

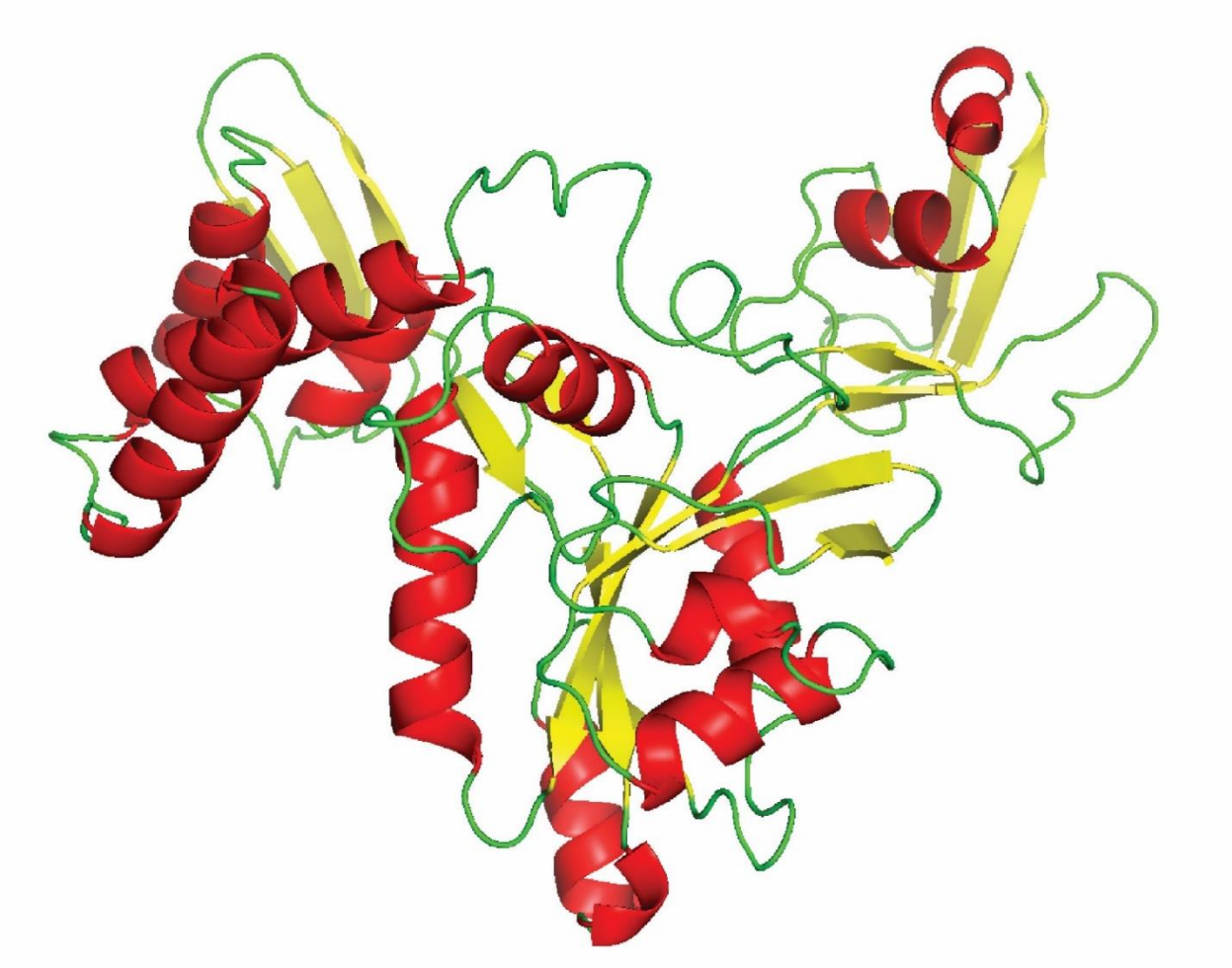


Fig. 1. 3D model of Human DRG2 protein visualized in PyMol.

Here in ribbon presentation, helix, sheet and loop regions are shown in red, yellow and green color respectively.

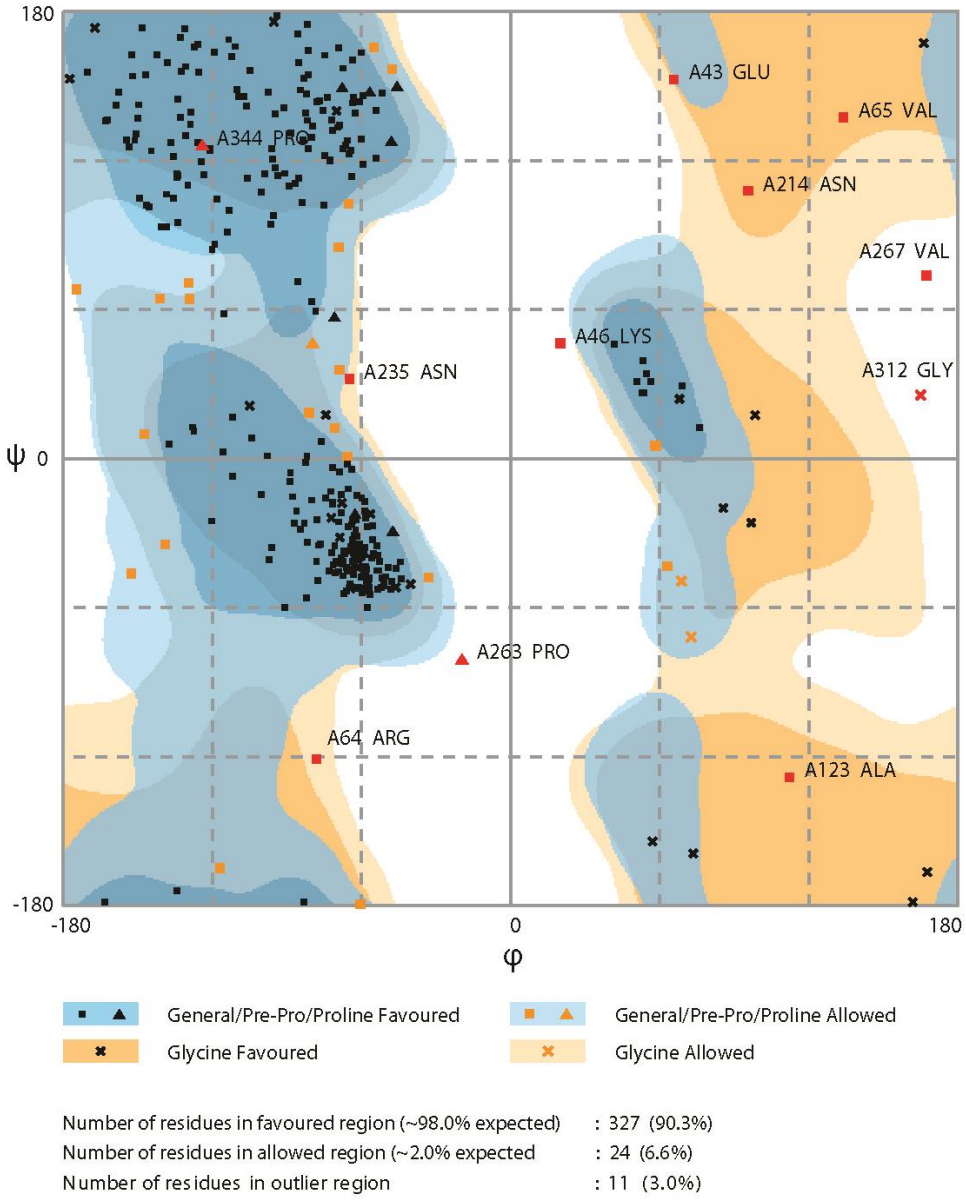


Fig. 2. Ramachandran validation plot of DRG2 protein model.

In this plot, colored region covered the favored and allowed region. The white area defined as outlier region.

Table 1: Top metabolites which showed binding energy more than -7.0 kj/mol.

No.	HMDB ID	Metabolite name	Binding energy with DRG2 (kj\mole)	Pathway involved
1	HMDB02829	Androsterone glucuronide	-10.2	Androstenedione Metabolism
2	HMDB04484	Etiocholanolone glucuronide	-10.2	Androstenedione Metabolism
3	HMDB04483	Estrone glucuronide	-10.1	Estrone Metabolism
4	HMDB06224	17-beta-Estradiol-3-glucuronide	-10.0	Androgen and Estrogen Metabolism, Aromatase deficiency, 17-Beta hydroxysteroid Dehydrogenase III Deficiency
5	HMDB00221	NADPH	-9.9	Vast amount of pathway including Androgen and Estrogen Metabolism, Androstenedione Metabolism
6	HMDB03193	Testosterone glucuronide	-9.8	Androgen and Estrogen Metabolism, Aromatase deficiency, 17-Beta hydroxysteroid Dehydrogenase III Deficiency
7	HMDB00374	17-Hydroxy-progesterone	-9.2	Androgen and Estrogen Metabolism, Aromatase deficiency
8	HMDB02759	Androsterone sulfate	-9.2	Androgen and Estrogen Metabolism
9	HMDB01032	Dehydroepiandrosterone sulfate	-9.1	Androgen and Estrogen Metabolism, Aromatase deficiency, 17-Beta hydroxysteroid Dehydrogenase III Deficiency
10	HMDB00757	Glycogen	-9.1	Starch and sucrose metabolism, Glycogen synthetase deficiency
11	HMDB00936	Uroporphyrin I	-9.0	Acute intermittent porphyria, Hereditary Coproporphyria (HCP), Porphyria Variegata (PV), Porphyrin Metabolism

No.	HMDB ID	Metabolite name	Binding energy with DRG2 (kj\mole)	Pathway involved
12	HMDB00418	18-Hydroxy-cortisol	-8.9	Not available.
13	HMDB00449	5a-Tetrahydro-corticosterone	-8.8	Not available.
14	HMDB00546	Epietiocholanolone	-8.5	Not available.
15	HMDB00015	Cortexolone	-8.3	Steroidogenesis
16	HMDB00058	Cyclic AMP	-8.3	Gout or Kelley-Seegmiller Syndrome Purine Metabolism
17	HMDB00494	Stigmastanol	-8.3	Not available.
18	HMDB00031	Androsterone	-8.1	Androstenedione Metabolism
19	HMDB00063	Cortisol	-8.1	Steroidogenesis
20	HMDB00363	17a-Hydroxy-pregnenolone	-8.1	Androgen and Estrogen Metabolism Steroidogenesis
21	HMDB00416	17-Hydroxy-pregnenolone sulfate	-8.1	Not available
22	HMDB00551	Etiocholanediol	-7.9	Not available
23	HMDB00037	Aldosterone	-7.8	Steroidogenesis, Glucose Transporter Defect (SGLT2)
24	HMDB00053	Androstenedione	-7.8	Androgen and Estrogen Metabolism Androstenedione Metabolism

No.	HMDB ID	Metabolite name	Binding energy with DRG2 (kj\mole)	Pathway involved
25	HMDB00241	Protoporphyrin IX	-7.8	Porphyrin Metabolism
26	HMDB00550	5-Andro-stenetriol	-7.8	Not available
27	HMDB00054	Bilirubin	-7.7	Porphyrin Metabolism
28	HMDB00295	Uridine 5'-diphosphate	-7.6	Androgen and Estrogen Metabolism Androstenedione Metabolism
29	HMDB00526	5alpha-Tetra-hydrocortisol	-7.6	Not available
30	HMDB00570	Coproporphyrin III	-7.6	Porphyrin Metabolism
31	HMDB00151	Estradiol	-7.5	Androgen and Estrogen Metabolism
32	HMDB00253	Pregnenolone	-7.5	Steroidogenesis
33	HMDB00288	Uridine 5'-monophosphate	-7.5	Lactose Synthesis
34	HMDB00653	Cholesterol sulfate	-7.5	Not available
35	HMDB00234	Testosterone	-7.3	Androgen and Estrogen Metabolism Androstenedione Metabolism
36	HMDB00707	4-Hydroxy-phenylpyruvic acid	-7.1	Tyrosine Metabolism
37	HMDB00032	7-Dehydrocholesterol	-7.0	Steroid Biosynthesis

No.	HMDB ID	Metabolite name	Binding energy with DRG2 (kj\mole)	Pathway involved
38	HMDB01273	Guanosine triphosphate (GTP)	-6.9	Various metabolism including gluconeogenesis, glutaminolysis and cancer, glycogenosis
39	HMDB01201	Guanosine Diphosphate (GDP)	-6.8	Various metabolism including gluconeogenesis, glutaminolysis and cancer, glycogenosis

GTP and GDP metabolites are also included. The table included the metabolites HMDB ID, name, binding energy from autodock vina analysis and common pathways they are involved, collected from HMDB database

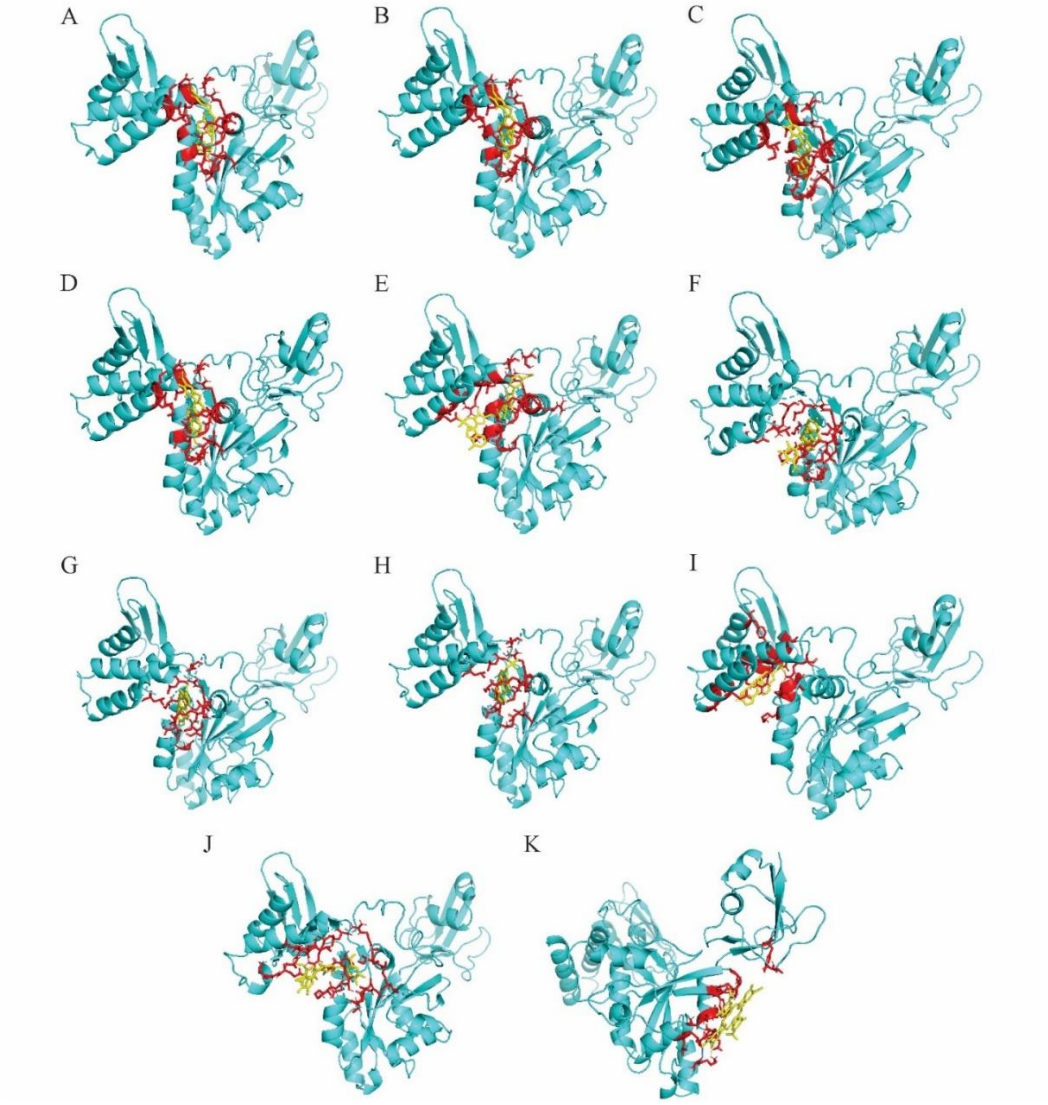


Fig. 3. 3D visualization of docking of top listed metabolites against DRG2 protein model.

In every panel each metabolite, active docking sites in DRG2 and the DRG2 model are shown in yellow, red and cyan color respectively. (A) Androsterone glucuronide, (B) Etiocholanolone glucuronide, (C) Estrone glucuronide, (D) 17-beta-Estradiol-3-glucuronide, (E) NADPH, (F) Testosterone glucuronide, (G) 17-Hydroxy-progesterone, (H) Androsterone sulfate, (I) Dehydroepiandrosterone sulfate, (J) Glycogen, (K) Uroporphyrin I.

Table 2: The active binding residues within DRG2 which were found to be involved in interaction with top 11 metabolites are listed.

No.	HMDB ID	Metabolite name	Active binding residues
1	HMDB02829	Androsterone glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165
2	HMDB04484	Etiocholanolone glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165
3	HMDB04483	Estrone glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165
4	HMDB06224	17-beta-Estradiol-3-glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162
5	HMDB00221	NADPH	I7, S8, R38, L41, E43, S45, E121, G122, A123, A124, G126, K127, G128, R129, G130, R131, I134, K161, E162, S165, V166
6	HMDB03193	Testosterone glucuronide	L4, E11, R38, L41, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, K161, E162, S165
7	HMDB00374	17-Hydroxy-progesterone	L4, L41, E43, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166
8	HMDB02759	Androsterone sulfate	L4, L41, E43, G118, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166
9	HMDB01032	Dehydroepiandrosterone sulfate	E11, I14, A15, K31, L34, A35, R38, A39, K161, E164, S165, V166, G167, Y210, D226, I229, D230, V233, R236
10	HMDB00757	Glycogen	L4, E5, I7, S8, E11, K12, R38, L41, L97, G118, I119, I120, E121, A123, A124, G130, R131, V133, I134,
11	HMDB00936	Uroporphyrin I	L81, S84, E105, Y106, K107, G108, K273, L276, D277, Y278, L280, E281, Y293, T304

No.	HMDB ID	Metabolite name	Active binding residues
12	HMDB01273	Guanosine triphosphate	L41, E43, S45, K46, S47, S50, K51, F55, D56, A123, A124, G126, K127, G126, G130, R131, Q132, I134, A135
13	HMDB01201	Guanosine Diphosphate	M1, L4, L41, S45, K46, S47, S50, K51, F55, D56, A124, K127, G128, R131, Q132

*GTP and GDP are also included.

**Active residues are defined by their one-letter abbreviation of amino acid name and number of that amino acid in DRG2 amino acid sequence.

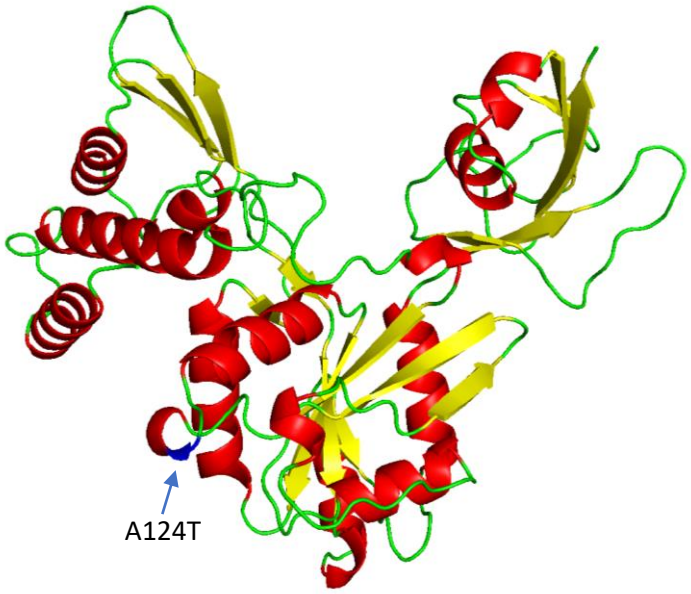
Table 3: The selected SNP of DRG2 protein metabolites from Biomuta database.

HMDB ID	Metabolite name	Active binding residues	Selected SNP of DRG2 include active residues
HMDB02829	Androsterone glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165	DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB04484	Etiocholanolone glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165	DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB04483	Estrone glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165	DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB06224	17-beta-Estradiol-3-glucuronide	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162	DRG2 A124T, DRG2 A124V
HMDB00221	NADPH	I7, S8, R38, L41, E43, S45, E121, G122, A123, A124, G126, K127, G128, R129, G130, R131, I134, K161, E162, S165, V166	DRG2 R38L, DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB03193	Testosterone glucuronide	L4, E11, R38, L41, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, K161, E162, S165	DRG2 R38L, DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB00374	17-Hydroxyprogesterone	L4, L41, E43, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166	DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB02759	Androsterone sulfate	L4, L41, E43, G118, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166	DRG2 A124T, DRG2 A124V, DRG2 S165F
HMDB01032	Dehydroepiandrosterone sulfate	E11, I14, A15, K31, L34, A35, R38, A39, K161, E164, S165, V166, G167, Y210, D226, I229, D230, V233, R236	DRG2 A15S, DRG2 R238L, DRG2 A39P, DRG2 S165F, DRG2 D230N
HMDB01273	Guanosine triphosphate	L41, E43, S45, K46, S47, S50, K51, F55, D56, A123, A124, G126,	DRG2 D56Y, DRG2 A124T, DRG2 A124V

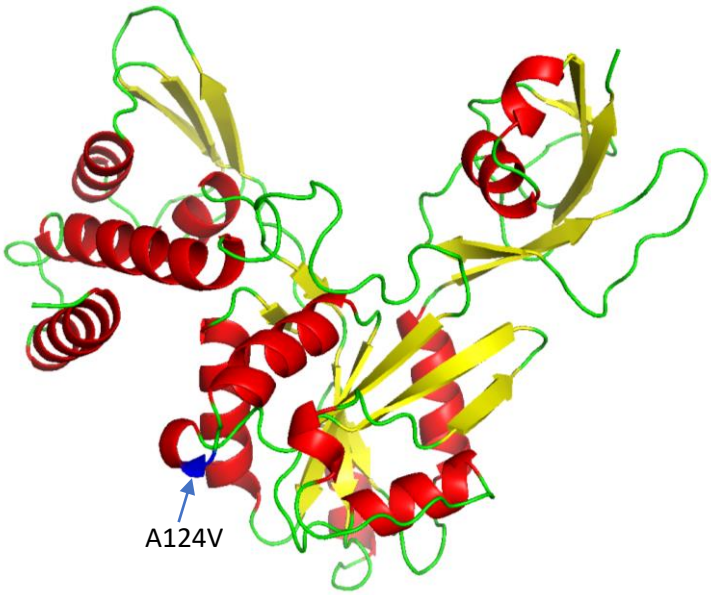
HMDB ID	Metabolite name	Active binding residues	Selected SNP of DRG2 include active residues
HMDB01201	Guanosine Diphosphate	K127, G126, G130, R131, Q132, I134, A135 M1, L4, L41, S45, K46, S47, S50, K51, F55, D56, A124, K127, G128, R131, Q132	DRG2 D56Y, DRG2 A124T, DRG2 A124V

The selected SNP of DRG2 protein metabolites from Biomuta database, which are changed in the residues involved in binding with top metabolites. GTP and GDP are also included.

A



B



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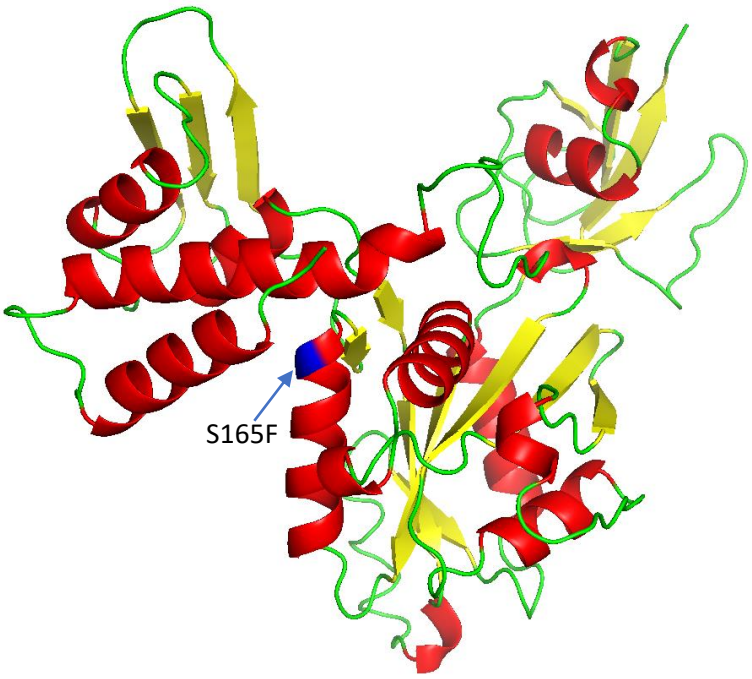


Fig. 4. 3D model of Human DRG2 Variants visualized in PyMol.

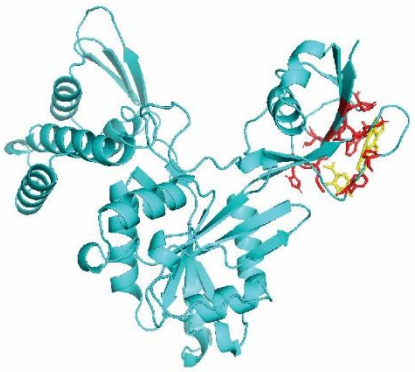
Here in ribbon presentation, helix, sheet and loop regions are shown in red, yellow and green color respectively. Blue color represented the changed amino acid residue. (A) DRG2 A124T variant, (B) DRG2 A124V variant, (C) DRG2 S165F variant.

Table 4: Top 39 metabolites binding energy value with DRG2 variants.

No.	HMDB ID	Metabolite name	Binding energy with DRG2 and DRG2 variants(kj\mole)			
			DRG2	DRG2 A124T	DRG2 A124V	DRG2 S165F
1	HMDB02829	Androsterone glucuronide	-10.2	-9.0	-9.8	-8.8
2	HMDB04484	Etiocholanolone glucuronide	-10.2	-9.7	-10.2	-9.7
3	HMDB04483	Estrone glucuronide	-10.1	-9.3	-9.9	-9.4
4	HMDB06224	17-beta-Estradiol-3-glucuronide	-10.0	-9.4	-9.9	-9.2
5	HMDB00221	NADPH	-9.9	-10.7	-10.0	-10.3
6	HMDB03193	Testosterone glucuronide	-9.8	-10.0	-10.1	-9.2
7	HMDB00374	17-Hydroxy-progesterone	-9.2	-9.7	-8.5	-8.5
8	HMDB02759	Androsterone sulfate	-9.2	-8.5	-8.4	-8.2
9	HMDB01032	Dehydroepiandrosterone sulfate	-9.1	-8.1	-8.6	-8.0
10	HMDB00757	Glycogen	-9.1	-9.1	-9.6	-9.4
11	HMDB00936	Uroporphyrin I	-9.0	-9.7	-9.8	-10.0
12	HMDB00418	18-Hydroxy-cortisol	-8.9	-9.3	-10.0	-9.3
13	HMDB00449	5a-Tetrahydro-corticosterone	-8.8	-9.6	-8.4	-8.7
14	HMDB00546	Epietiocholanolone	-8.5	-9.0	-8.4	-8.3
15	HMDB00015	Cortexolone	-8.3	-9.1	-8.6	-8.3
16	HMDB00058	Cyclic AMP	-8.3	-7.3	-7.4	-7.0
17	HMDB00494	Stigmastanol	-8.3	-8.0	-8.4	-8.2
18	HMDB00031	Androsterone	-8.1	-9.3	-8.4	-8.4
19	HMDB00063	Cortisol	-8.1	-8.9	-9.4	-8.7
20	HMDB00363	17a-Hydroxy-pregnenolone	-8.1	-9.0	-8.9	-8.3

No.	HMDB ID	Metabolite name	Binding energy with DRG2 and DRG2 variants (kj\mole)			
			DRG2	DRG2 A124T	DRG2 A124V	DRG2 S165F
21	HMDB00416	17-Hydroxy-pregnenolone sulfate	-8.1	-9.3	-8.9	-8.8
22	HMDB00551	Etiocholanediol	-7.9	-9.0	-8.4	-8.4
23	HMDB00037	Aldosterone	-7.8	-8.4	-8.1	-8.4
24	HMDB00053	Androstenedione	-7.8	-8.7	-8.1	-8.2
25	HMDB00241	Protoporphyrin IX	-7.8	-8.6	-8.5	-7.9
26	HMDB00550	5-Andro-stenetriol	-7.8	-9.0	-8.5	-8.7
27	HMDB00054	Bilirubin	-7.7	-6.4	-7.5	-6.9
28	HMDB00295	Uridine 5'-diphosphate	-7.6	-7.6	-8.4	-7.8
29	HMDB00526	5alpha-Tetra-hydrocortisol	-7.6	-8.5	-8.4	-8.4
30	HMDB00570	Coproporphyrin III	-7.6	-8.5	-8.2	-7.7
31	HMDB00151	Estradiol	-7.5	-8.4	-7.8	-7.6
32	HMDB00253	Pregnenolone	-7.5	-8.7	-8.5	-8.3
33	HMDB00288	Uridine 5'-monophosphate	-7.5	-6.9	-7.5	-7.0
34	HMDB00653	Cholesterol sulfate	-7.5	-7.9	-8.1	-8.6
35	HMDB00234	Testosterone	-7.3	-9.0	-8.1	-8.2
36	HMDB00707	4-Hydroxy-phenylpyruvic acid	-7.1	-6.0	-5.9	-5.7
37	HMDB00032	7-Dehydrocholesterol	-7.0	-7.8	-8.3	-7.8
38	HMDB01273	Guanosine triphosphate (GTP)	-6.9	-6.9	-7.9	-7.3
39	HMDB01201	Guanosine Diphosphate (GDP)	-6.8	-7.2	-7.7	-7.6

A



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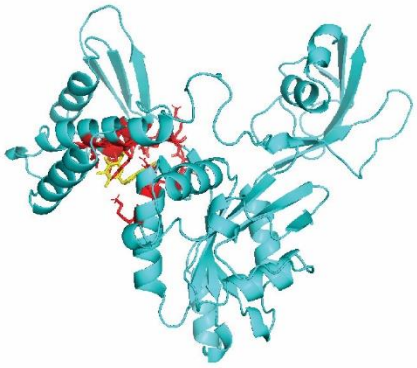

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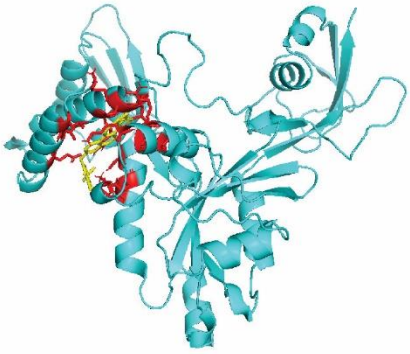

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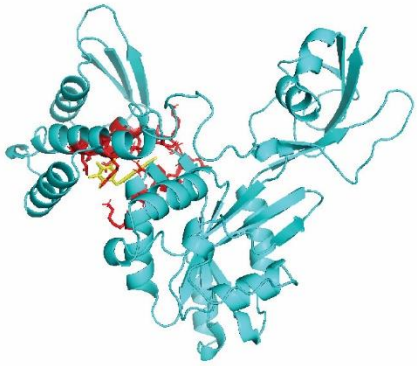

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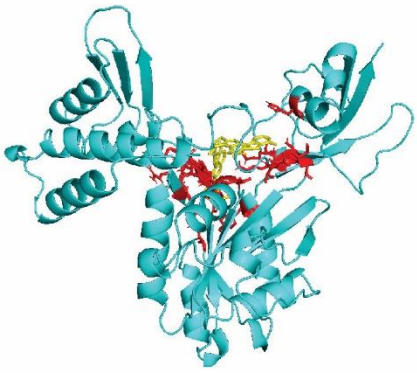

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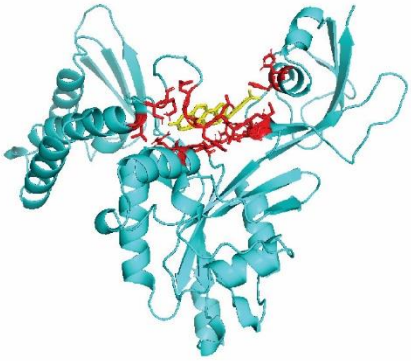

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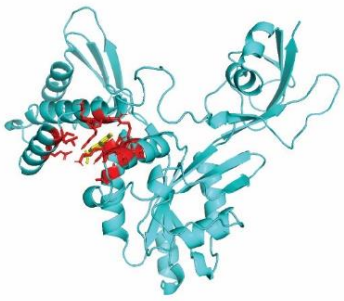

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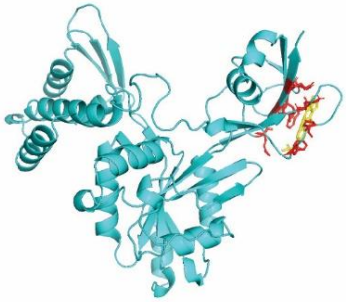

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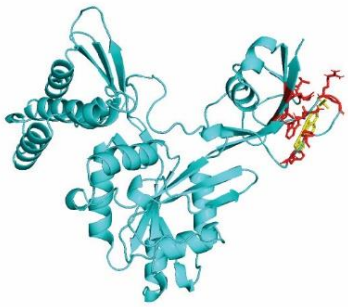

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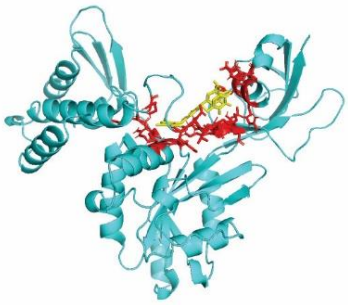

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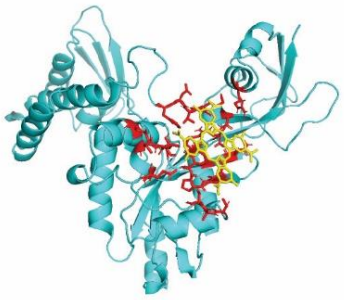

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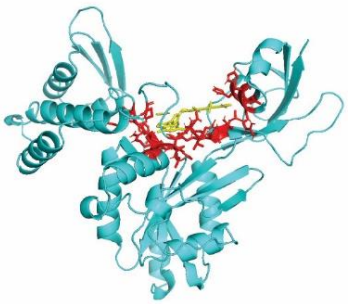

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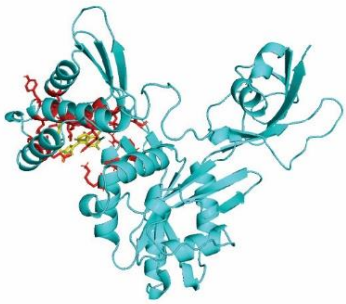

Fig. 5. 3D visualization of docking of top listed metabolites against DRG2 A124T variant.

In every panel each metabolite, active docking sites in DRG2 A124T and the DRG2 A124T model are shown in yellow, red and cyan color respectively. (A) Androsterone glucuronide, (B) Etiocholanolone glucuronide, (C) Estrone glucuronide, (D) 17-beta-Estradiol-3-glucuronide, (E) NADPH, (F) Testosterone glucuronide, (G) 17-Hydroxy-progesterone, (H) Androsterone sulfate, (I) Dehydroepiandrosterone sulfate, (J) Glycogen, (K) Uroporphyrin I, (L) GTP, (M) GDP.

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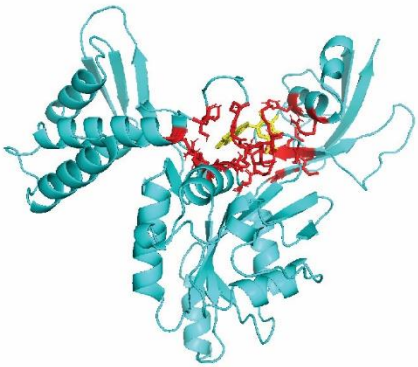

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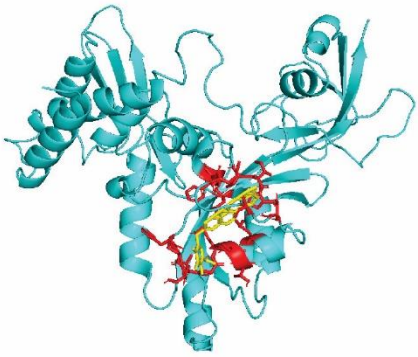

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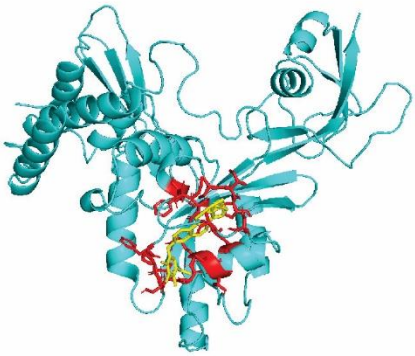

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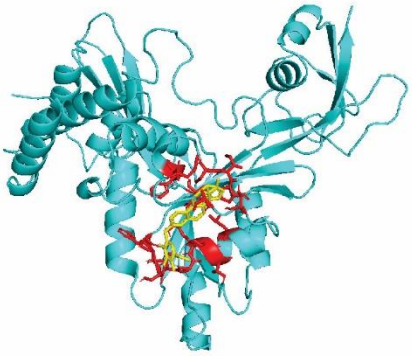

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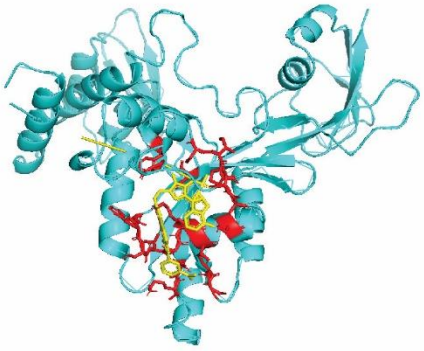

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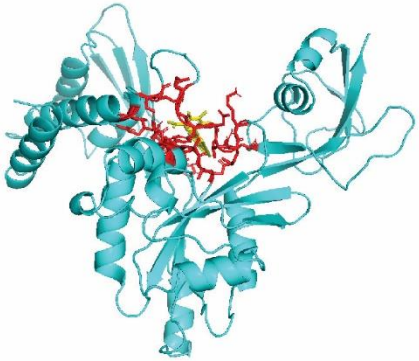

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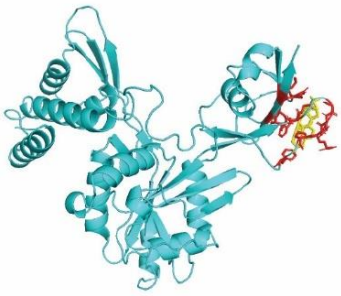

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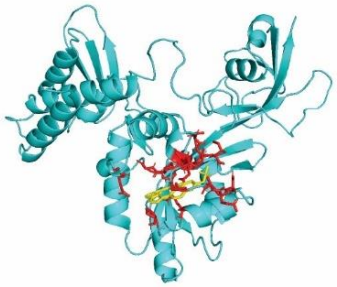

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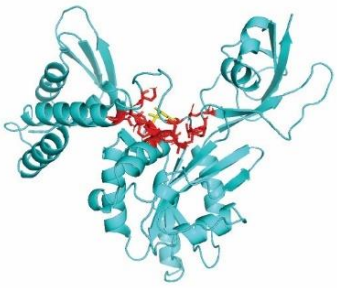

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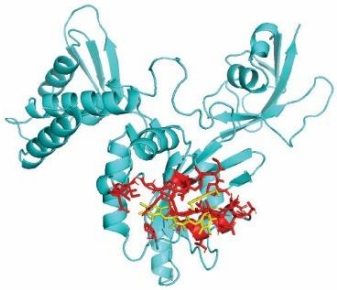

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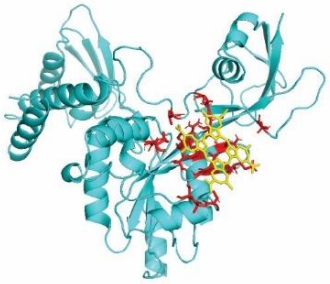

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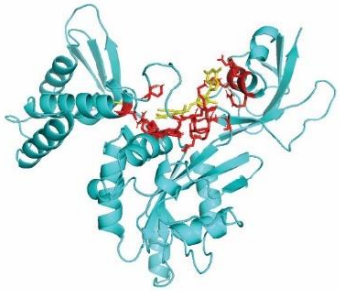

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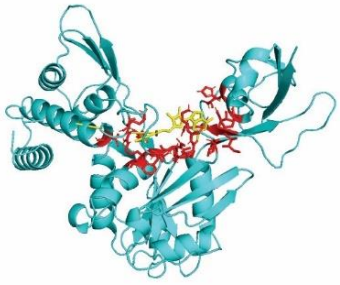
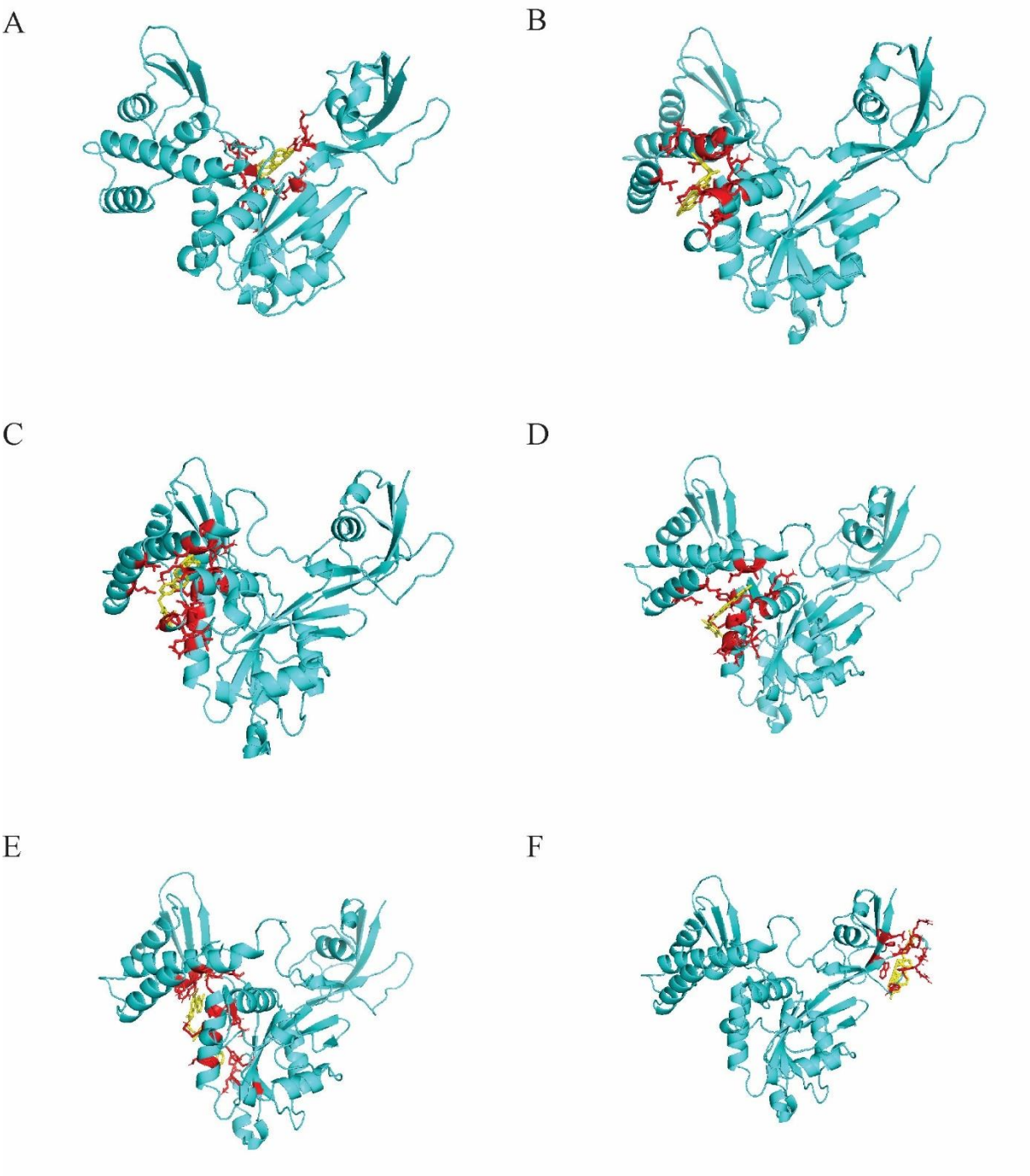
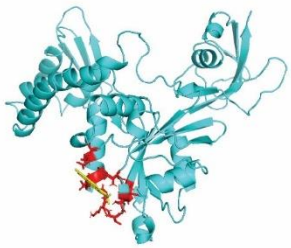


Fig. 6. 3D visualization of docking of top listed metabolites against DRG2 A124V variant.

In every panel each metabolite, active docking sites in DRG2 A124V and the DRG2 A124V model are shown in yellow, red and cyan color respectively. (A) Androsterone glucuronide, (B) Etiocholanolone glucuronide, (C) Estrone glucuronide, (D) 17-beta-Estradiol-3-glucuronide, (E) NADPH, (F) Testosterone glucuronide, (G) 17-Hydroxy-progesterone, (H) Androsterone sulfate, (I) Dehydroepiandrosterone sulfate, (J) Glycogen, (K) Uroporphyrin I, (L) GTP, (M) GDP.


A
B
C
D
E
F

G



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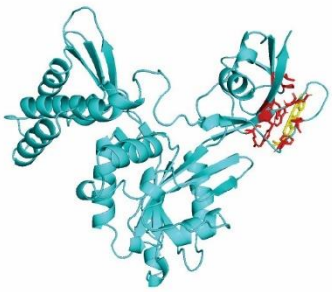

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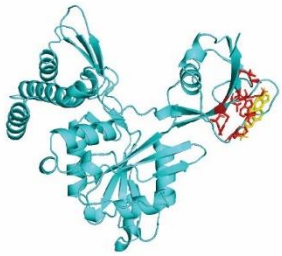

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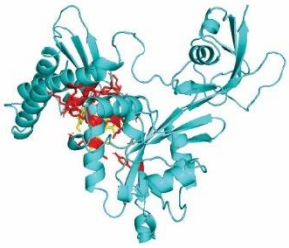

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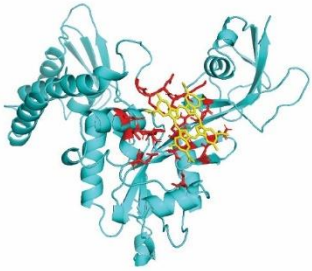

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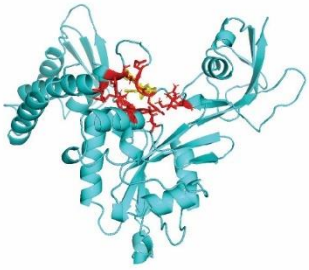

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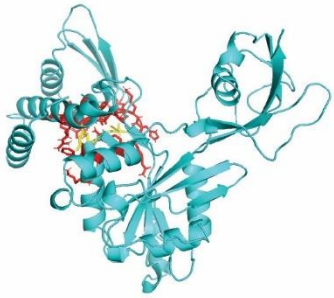

Fig. 7. 3D visualization of docking of top listed metabolites against DRG2 S165F variant.

In every panel each metabolite, active docking sites in DRG2 S165F and the DRG2 S165F model are shown in yellow, red and cyan color respectively. (A) Androsterone glucuronide, (B) Etiocholanolone glucuronide, (C) Estrone glucuronide, (D) 17-beta-Estradiol-3-glucuronide, (E) NADPH, (F) Testosterone glucuronide, (G) 17-Hydroxy-progesterone, (H) Androsterone sulfate, (I) Dehydroepiandrosterone sulfate, (J) Glycogen, (K) Uroporphyrin I, (L) GTP, (M) GDP.

Table 5: The active binding residues within each DRG2 variant which were found to be involved in interaction with top 11 metabolites, GTP and GDP are listed.

No.	HMDB ID	Active binding residues in DRG2 and DRG2 variants			
		DRG2	**DRG1 A124T**	**DRG2 A124V**	**DRG2 S165F**
1	HMDB02829	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165	K295, P301, F303, W336, T338, P344, H355, E356, V358, Q360	L42, S45, K46, E53, G54, F55, D56, V57, M58, K59, G61, A135, R138, T139, D141, V237, L289, I308, L309, R310, H317, R321	N171, K172, H173, Y238, M239, P240, P263, N264, S264, Y286, L287, A288, R310, K311, G312
2	HMDB04484	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165	K31, L34, A35, R38, K161, E164, R169, K172, H173, K174, P175, E221, S224, D226, E227, I229, D230, R236	F70, P71, S72, V73, G74, K75, S76, T77, L79, S87, A89, E93, F94, C99, I100, P101, D115,	M1, G2, L4, Q40, L41, L42, P44, Q125, K127, G128, R131, I134, E162, F165,
3	HMDB04483	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165	M1, L4, I7, E11, R38, L41, L42, G122, K127, G130, R131, I134, K160, E161, S165	F70, P71, S72, V73, G74, K75, S76, T77, L79, S87, A89, E93, F94, C99, I100, P101, D115,	L4, E11, L41, L42, P44, I120, E121, G122, A123, Q125, K127, G130, R131, I134, L158, K161, E162, F165,
4	HMDB06224	M1, I3, L4, Q40, L41, E43, G118, I119, I120, E121, G122, A123, A124, G126, K127, G128, G130, R131, I134, E162	K31, L34, A35, R38, K161, E169, K172, H173, K174, P175, E221, S224, D226, E227, I229, D230, R236	F70, P71, S72, V73, G74, K75, S76, T77, L79, S87, A89, E93, F94, C99, I100, P101, D115,	L4, E11, L41, L42, P44, I120, E121, G122, A123, Q125, K127, G130, R131, I134, S160, L158, K161, E162, F165,
5	HMDB00221	I7, S8, R38, L41, E43, S45, E121, G122, A123, A124, G126, K127, G128, R129, G130, R131, I134, K161, E162, S165, V166	V57, M58, K59, S60, G61, R138, T139, R169, N171, K172, H173, R236, V237, Y238, P240, N264, E285, Y286, R310, H317	F70, P71, S72, V73, G74, K75, S76, T77, S80, A86, S87, F94, C99, I100, P101, D115, P117, N246, K247, C270, G271	K30, R38, R156, S157, E160, K161, E164, R169, L170, N171, K171, P175, S224, D226, E227, D230, R236, Y243, Y245, K262, P263,
6	HMDB03193	L4, E11, R38, L41, I119, I120, E121, G122, A123, A124, G126, K127, G128,	L42, S45, K46, E53, G54, F55, D56, V57, M58, K59, S60, A135, R138, T139, R310,	L42, S45, K46, E53, G54, F55, D56, V57, M58, K59, A135, R138, T139, A140, D141, H173, R235, V236, Y237, R310,	K295, K296, R297, Q299, R300, P301, F303, L334, W336, P344, V358, Q360
7	HMDB00374	L4, L41, E43, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166	M1, L4, I7, E11, R38, L41, K127, G128, G130, R131, I134, E162, S165, V166,	K295, G298, Q299, R300, P301, F303, L334, W336, P344, V358, Q360	F70, S72, V73, E121, L147, D148, T150, K151, G152, V154, Q155, L158,

No.	HMDB ID	Active binding residues in DRG2 and DRG2 variants			
		DRG2	**DRG1 A124T**	**DRG2 A124V**	**DRG2 S165F**
8	HMDB02759	L4, L41, E43, G118, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166	K295, P301, F303, W336, P344, E356, V358, Q360	P71, K75, S87, E88, A89, S91, E93, F94, T98, C99, I100, D115, P117, G118, E121,	Y293, K295, P301, F303, L334, W336, P344, E356, V358
9	HMDB01032	E11, I14, A15, K31, L34, A35, R38, A39, K161, E164, S165, V166, G167, Y210, D226, I229, D230, V233, R236	K295, R297, G298, P301, F303, L334, W336, P344, V358, Q360	L42, K46, F55, V57, M58, A135, R138, T139, A140, D141, R236, V237, Y238, R310,	Y293, K295, P301, F303, L334, W336, P344, E356, V358
10	HMDB00757	L41, E43, S45, K46, S47, S50, K51, F55, D56, A123, A124, G126, K127, G126, G130, R131, Q132, I134, A135	K46, F55, D56, V57, M58, K59, S60, G61, A135, R137, T138, R236, V237, L309, R310, E316, H317, H320, R321, R324	P71, K75, S76, T77, L79, S80, T83, T85, A86, S87, E88, A89, A90, S91, Y92, E93, F94, C99, I100, P101, D115, P117, G118, I120, E121, V124,	K30, R38, E160, K161, E164, F165, G167, R169, L170, N171, K172, H173, K174, P175, E221, S224, D226, E227, D230, R236, Y243,
11	HMDB00936	M1, L4, L41, S45, K46, S47, S50, K51, F55, D56, A124, K127, G128, R131, Q132	F55, D56, V57, M58, G61, D62, R64, T 85, A86, S87, E93, T96, L97, T98, I100, P101, G102, V103, N110, Q112, Q132, R321	V57, S60, G61, D62, R64, T85, A86, S87, L97, T98, I100, G102, V103, E105, N110, Q112, D305	S87, E93, T96, L97, T98, I100, V103, N110,Q112, Q132,
12	HMDB01273	L4, L41, E43, G118, I119, E121, A123, A124, G126, K127, G128, G130, R131, I134, E162, S165, V166	K47, F55, D56, V57, K59, S60, A135, R138, T139, A140, N235, R236, V237, Y238, L309, R310, H317, V318, H320, R321	L41, K46, F55, D56, V57, M58, K59, G61, A135, R1138, T139, D141, V237, R310, E316, H317, H320, R321	L42, S45, K46, E53, G54, F55, V57, K59, A135, R138, T139, A140, G234, N235, R236, V237, Y238, R310,
13	HMDB01201	E11, I14, A15, K31, L34, A35, R38, A39, K161, E164, S165, V166, G167, Y210, D226, I229, D230, V233, R236	E11, I14, K19, Y25, L27, G28, K31, L34, A35, R37, K161, E164, R169, L202, P225, D226, I229, D230, R236	L41, K46, F55, D56, V57, M58, K59, G61, A135, R138, T139, D141, V237, L288, I308, L309, R310, A313, H317, R321,	K31, A35, R38, K161, E164, F165, R169, N171, K172, H173, K174, P175, Q197, E221, S224, D226, E227, I228, D229, R236,

4. DISCUSSION

Cells can recognize the changes in cellular metabolism by metabolite-interacting proteins and respond to the changes in metabolism using the proteins. Thus, it is possible to predict that proteins interacting with metabolites may play essential roles in modulating metabolite signaling. DRG2 is a GTP-binding protein. Until now, there have been no reports on the role of DRG2 in metabolite signaling. We here report the first analysis of DRG2-metabolites interactions and binding sites using in silico docking analysis. The analysis resulted in 37 metabolites with binding affinity with DRG2 higher than GTP. Most of the 37 metabolites belonged to steroid hormone metabolites, indicating that DRG2 may recognize these steroid hormone metabolites and regulate these steroid hormones signaling pathway.

Steroid hormones bind to their respective steroid hormone receptors (SR) and generate a wide array of essential cellular and physiological responses. Traditionally, steroid hormones-receptor complexes are known to function in the cell nucleus as transcription factors to selectively modulate gene expression. Ligand-activated SRs dissociate from hsp90 chaperone complexes in the cytoplasm and enter the nucleus where they bind to specific DNA sequences: hormone response elements (HREs). SRs interact with coregulator proteins (coactivators and corepressors) as well as chromatin remodeling complexes to regulate target gene expression [43]. Besides this traditional genomic function, steroid hormones can bind to membrane-associated receptors and activate several signal transduction pathways in the cytoplasm [44, 45]. Dysregulation of SR-target gene expression, in some instances, have significant consequences on the proliferation and/or death of cells that affect tissue structure and remodeling (animal development), as well as a multitude of disease processes, such as cancer [46-50]. In our study, we found that DRG2 may interact with

steroid hormone metabolites. This suggests that DRG2, after binding with steroid hormone metabolites, may play some roles in the regulation of gene expression by regulating dissociation of hsp90 from SR or acting as coregulators of SR. By using, in silico docking analysis, we determined amino acid residues of DRG2 involved in the interaction with steroid hormone metabolites. We also screened for the single nucleotide variation within DRG2 by BioMuta analysis and found that five amino acid residues R38, D56, A124, S165, and D230 of DRG2 responsible for the binding with the steroid hormone metabolites were substituted by other amino acids in human cancer cells. We did not determine whether these alterations of DRG2 affect the binding of steroid hormone metabolites. However, it is possible that these amino acid substitutions in DRG2 may alter the binding affinity with steroid hormone metabolites and may cause dysregulation in the expression of steroid hormone target genes, which can lead to tumor growth and/or metastasis. Further studies are required to confirm the interaction between DRG2 and metabolites selected by in silico method and to confirm whether amino acid substitutions in DRG2 found in the human cancer cells affect the metabolite binding to DRG2 and the expression of steroid hormone target genes in cancer cells.

In summary, we here analyzed 420 metabolites for DRG2 binding by in silico method and found 37 metabolites showing binding affinity with DRG2 higher than GTP (-6.9 kj\mole). Most of the metabolites with high binding affinity belonged to the steroid hormone metabolites. By BioMuta analysis, we found that some human cancer cells have amino acid substitution in steroid hormone metabolite binding site of DRG2. Considering the role of steroid hormone in the regulation of gene expression and cancer growth, it is possible that amino acid substitution in the steroid hormone metabolite-binding site of DRG2 may cause change in the expression of steroid hormone target genes and affect cancer growth. Collectively, even though it remained to be determined by

validation experiments, the discovery of DRG2-interacting metabolites would extend our understanding of DRG2 function in steroid hormone metabolism and can be used to develop drugs targeting steroid hormone-related diseases.

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