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Research Article

IN SILICO ANALYSIS OF ACRAL PEELING SKIN SYNDROME: A PROTEOMIC APPROACH

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ABSTRACT

Objective: Acral peeling skin syndrome (APSS), a rare genetic disorder, indicated by the continuous blistering and shedding of the outer epidermal layers. Transglutaminase 5 (TGM5), a calcium-dependent TGM, present in the epidermis has been implicated as the cause of APSS. An attempt has been made to compare *in silico* the wild and mutant form of TGM5 and its implication on its interaction with involucrin (IVL).

Methods: Comparative modeling was performed using MAESTRO for proteins TGM5 and IVL using templates from the protein databank. Generated model was later refined using side chain refinement and loop refinement. Three-dimensional (3D) structure of TGM5 and IVL was analyzed in PROCHECK, VERIFY3D, and ERRAT was used to assess the reliability of the 3D model. IMPACT package from Schrödinger was used to generate a binding site for calcium ion which is essential for functioning of protein. Energy minimization for the modelled structures was performed using IMPACT module of Schrodinger. Subsequently, wild type and mutated models of TGM5 was used for performing docking studies with IVL.

Results: The structures for TGM5 and IVL were modeled and energy minimized using Schrödinger suite. Conserved calcium binding domain formed by three asparagine residues (N224, N226 and N229) and alanine (A221) corresponding to TGM3 was found in TGM5 at positions 226, 229, 231, and 234. Identification of probable active site for TGM5 was predicted using SiteMap program in Schrödinger. 17 cysteine residues are present in wild type structure of TGM5 and in mutated form G113C, the probability of forming an extra disulfide increases. With the mutation occurring at 113 position formation of disulfide bond between C113 and Cys306 increases manifold. This hypothesis was confirmed by the fact that root-mean-square distance value of energy minimized mutated TGM5 when compared to native TGM5 on aligning all 561 atoms was found to be 0.141 indicating a change of overall structure of protein.

Conclusion: The mutation G113C is increasing the dynamic nature of the protein to increase as the probability of the formation of disulfide bond increases.

Keywords: Skin, Acral peeling skin syndrome, Glutaminase, Involucrin, Mutation, Interaction.

INTRODUCTION

Acral peeling skin syndrome (APSS), a rare genetic disorder, indicated by the continuous blistering followed by shedding of the outer epidermal layers throughout life [1,2]. Rarely, symptoms such as erythema, vesicular lesions, fragile hair, and nail abnormalities have also been observed. APSS has been classified as non-inflammatory (Type A) and inflammatory (Type B). Transglutaminase 5 (TGM5), a calcium-dependent TGM, present in the epidermis [3,4] has been implicated as the cause of APSS. Sequence conservation has been found to be dominant in the primates (Fig. 1a). Gamma glutamyl-lysine isodipeptide bonds formed between adjacent polypeptides result in the contact between the granular and the corneum layer. Mutations in TGM5 have been found to disrupt the contacts between stratum granulosum and stratum corneum. Although many mutations such as T109M, G113C [2], p.M1T, p.L41P, p.L214CfsX15, and p.S604IfsX9 [5] have already been reported in TGM5, the structural basis of the loss of interaction has not been discussed.

Many proteins such as involucrin (IVL), loricrin, filaggrin, and small proline-rich proteins are involved in the formation of the cornified cell envelope in the biogenesis of the stratum corneum, the outermost, "dead" layer of the epidermis [6,7] (Fig. 1b). IVL, a protein synthesised in the stratum spinosum is cross-linked to integral membrane proteins in stratum granulosum like desmoplakin (Uniport) by TGM enabling the formation of insoluble envelope, which in turn acts as a template during assembly of the cornified envelope [8,9]. Thus, the interaction is mandatory for providing structural support to the cell and resisting invasion by micro-organisms. Till date, no study has indicated the structural basis of the interaction between TGM5 and any other protein. In this study, an attempt has been made to study the structural basis of interaction of TGM5 and IVL and the effect of mutation on the interactions between these two proteins and integrity of the epidermis.

METHODS

Materials

The three-dimensional (3D) structure of the protein sequence was designed using MAESTRO package of Schrödinger. The structure analysis and verification server SAVES (http://www.nihserver.mbi.ucla.edu/SAVES/) were used for performing the quality assessment of the modelled structures. SiteMap package was used to identify the binding cavities/active site. The molecular docking was performed using the ZDock 3.0.2 server, integrated with the Fast Fourier Transfer based docking method.

Methodology

Homology modeling

TGM5 sequence was retrieved from NCBI protein database (PDB) (Accession no: GenBank: AAI22860.1). Sequence and structure search was performed using NCBI basic local alignment search tool (BLAST) (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) against PDB. Comparative modeling usually starts by searching the PDB for similar protein structures using target sequence as the query. The template structure was selected on the basis of maximum query coverage and maximum identity. MAESTRO [10] was used to design the protein structure using 1L9M_A (human TGM 3 enzyme) as template. Hydrogen atoms were added to the model using Maestro interface (version 8.5; Schrodinger LLC, New York) based on an explicit all-atom model. Generated model was later refined using side chain refinement [11,12] and loop refinement. Similarly, 3D structure (3D) of the interacting protein, IVL from *Homo sapiens* (Accession no: NP_005538.2) was

modeled against template structure 3CVR_A (Ubiquitine ligase) based on its homology.

Quality assessment

The overall stereo chemical quality of the 3D structure of TGM5 and IVL was analyzed by Ramachandran plot using the program PROCHECK (http//www.biotech.ebi.ac.uk) [13,14]. In addition, VERIFY3D from NIH, MBI laboratory server [15] and ERRAT was used to assess the reliability of the 3-D model (nihserver.mbi.ucla.edu/ERRAT) [16]. The acceptability of the model was analyzed using the ProSA (https://www.prosa.services.came.sbg.ac.at/) for its overall model quality [17,18].

Ionization and active site prediction

It has been reported that calcium plays an important role in the differentiation and expression of keratinocytes [19,20] and is the important cofactor required for proper functioning of TGM2 and TGM3 proteins [21,22]. Hence, calcium ion was added as a cofactor to the protein to obtain the native functional form of TGM5. The IMPACT package from Schrödinger was used to generate binding using a grid margin of 6 Å, grid spacing of 0.7 Å, non-bonded cut-off of 20 Å, and phobic/philic point thresholds of −0.5/−8.0 Å. Top five sites were selected based on site score, size, and volume of the cavity.

Mutation and energy minimization

Energy minimization for the modeled structures was performed using IMPACT module of Schrodinger. *In silico* site directed mutagenesis was done to obtain the mutated form of the protein, TGM5. Primarily, mutations T109M and G113C were incorporated in the protein as molecular association studies has indicated that these two mutations are the ones affecting functioning. The mutated structure of the target protein was obtained by comparative modelling of the mutated target sequence, using the refined modeled structure of TGM5 as template. The model was analyzed for quality check and minimized to obtain the global minimum energy conformation. The modeled protein was subjected to energy minimization to remove the unwanted VdW energies and other structural clashes.

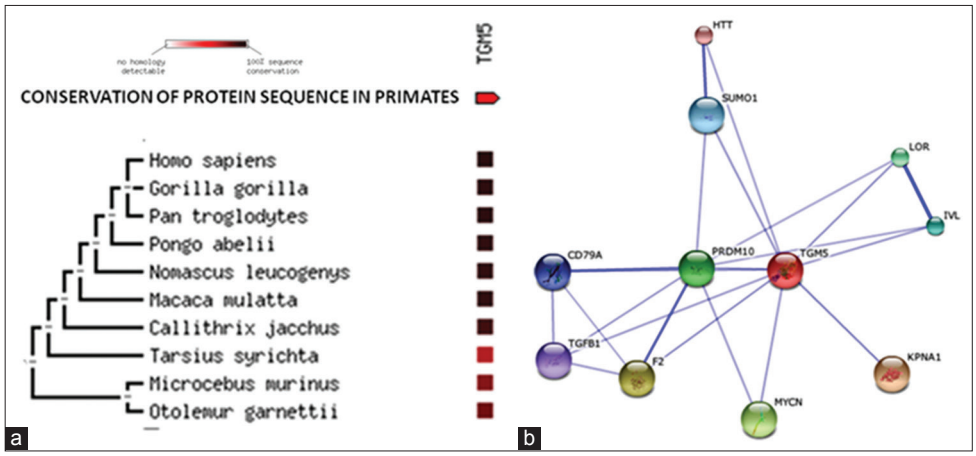

Fig. 1: (a) Sequence conservation compared for all the available transglutaminase 5 (TGM5) sequence present in the primates, (b) confidence view generated for TGM5 and its interacting partners IVL (involucrin), LOR (loricin), KPNA1 (Karyopherin), MYCN (v-myc myelocytomatosis), F2 (thrombin), PRDM10 (PR domain protein), SUMO1 (Ubiquitin like protein) and HTT (Huntingtin) (diagram developed using http://www.string-db.org/)

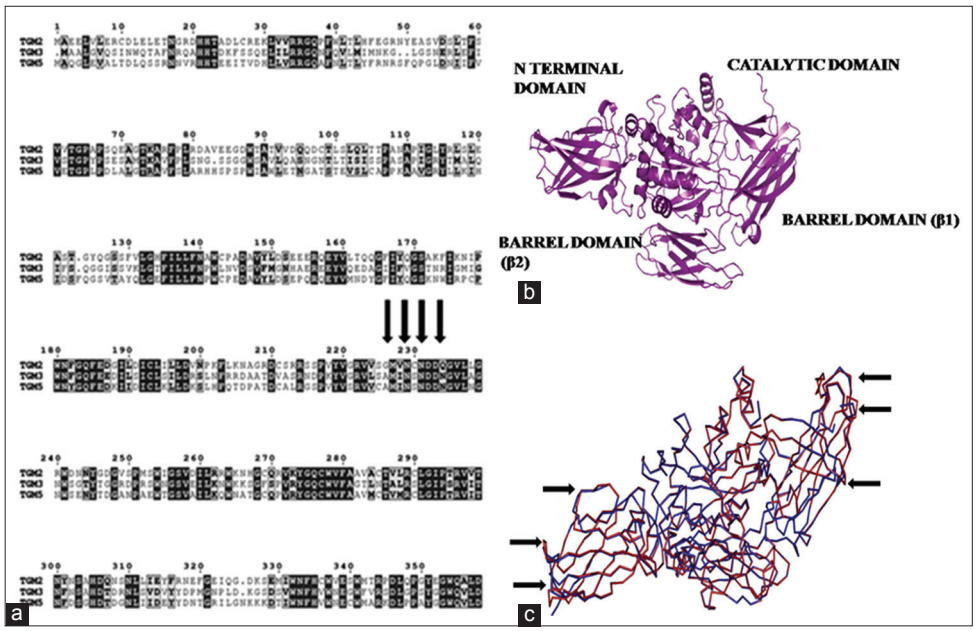

Fig. 2: (a) Sequence alignment of Transglutaminase 2 (TGM2), TGM3 and TGM5 from *Homo sapiens* with conserved residues highlighted by arrows at 226, 229, 231 and 234. Alignment made using ESPRIPT programme, (b) modelled structure of TGM5, (c) overlapped sequence of wtTGM5 (red) and mTGM5 (blue)

Molecular docking

The TGM5, mutated TGM5 and IVL docking was performed on ZDock protein-protein docking server (http://www.zdock.umassmed.edu/), where the complex models were analyzed using energy scoring function and FFT for quick calculations [18]. It also allows residue specific docking and blocking residues that show not be involved during the substrate interaction. The results are filtered based on a contact cut-off of 6 Å by default.

RESULTS

Homology modeling and structure validation of TGM5 and IVL

Template selection and sequence alignment for the target are the main criteria to decide the accuracy of the structure designed. Top three structure hits from NCBI protein data bank listed 1L9M (TGM 3) and 3LY6 (TGM 2). TGM3 was preferentially used as template on the basis on query coverage (98%) and percentage identity (45%)(Fig. 2a) and the alignment was juxtaposed to TGM5. Although, the presence of differences in sequence length and domain was present, proper alignment was done using structalign tool of the MAESTRO suite. The aligned sequence enabled prediction of the tertiary structure using energy based model method.

A total of nine amino acids were found to be in the disallowed regions in the 3D structure of TGM. The initial potential energy was recorded as −20028.867 kcal/mol and it reduced to −21035.344 kcal/mol on completion of energy minimization. The initial potential energy for modelled IVL was recorded as −15521 kcal/mol and it reduced to −22574.264 kcal/mol on energy minimization. The protein sequence identities, root mean square deviations, and Ramachandran plot statistics for the modeled structures of TGM5 and IVL are summarized in Table 1.

The modeled structure of TGM5 was analyzed using different tools like VERIFY 3D and PROSA. (Fig. 2b) VERIFY_3D analysis showed that 89.94% of TGM5 protein residues had an average 3D-1D score > 0.2 showing acceptable primary sequence to tertiary structure compatibility. Assessment of TGM5 and (IVL) structural quality in ProSA server indicated Z-score value −8.15 and −3.25 falls in the range of native conformations computed using X-ray crystallography method. Thus modeled residues of proteins fall in the negative energy minima region representing good structural quality. Thus based on the results, the dependability of the structures could be confirmed.

Functional analysis of interacting partners

Conserved calcium binding domain formed by three asparagine residues (N224, N226, and N229) and alanine (A221) corresponding to TGM3 was found in TGM5 at positions 226, 229, 231 and 234 (Fig. 3a) [23].

Identification of probable active site for TGM5 was predicted using SiteMap program in Schrödinger. Five top hits were selected and are highlighted in Fig. 4a and b. The predicted amino acids corresponding to glutaminase activity was found to be conserved at positions C274, H330, and D340 for the topmost hit selected.

On sequence analysis, it was found that there were 17 cysteine residues in the wild type structure of TGM5 and in mutated form G113C probability of forming an extra disulfide increases. With the mutation occurring at 113 position formation of disulfide bond between C113 and Cys306 increases manifold (Fig. 3b). This hypothesis was confirmed by the fact that root-mean-spuare distance value of energy minimized mutated TGM5 when compared to native TGM5 on aligning all 561 atoms was found to be 0.141 indicating a change of overall structure of protein.

Interaction analysis of TGM5 and IVL

Protein-protein docking was performed with the Z dock server using by default parameters.

Table 1: Structural validation of the modeled structures of TGM5 and IVL

Modelled protein	RMSD	Ramachandran plot statistics	
		Allowed region	Disallowed region
TGM5	0.2	96.34	2.67
IVL	0.1	96.26	3.73

RMSD: Root-mean-square distance, TGM5: Transglutaminase 5, IVL: Involucrin

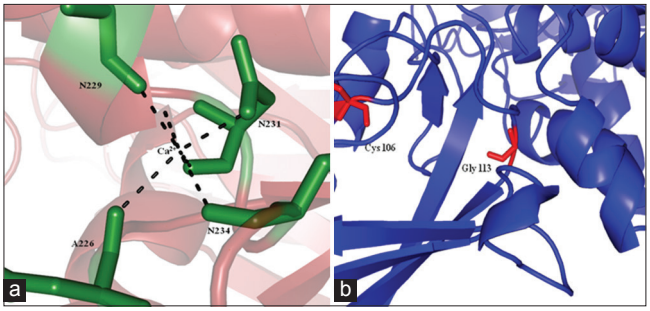

Fig. 3: (a) Ribbon representation of calcium binding pocket (highlighted in green) in Transglutaminase 5 (TGM5) from *Homo sapiens*, (b) site of mutation in mTGM5 where glycine is converted to cysteine at position 113

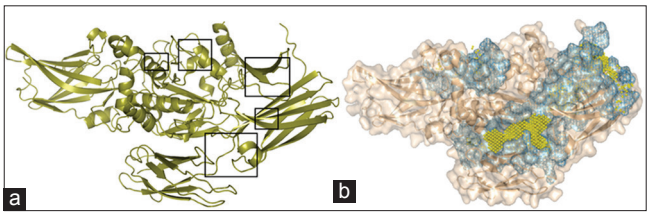

Fig. 4: (a) Ribbon representation of active site pockets (black boxes) in transglutaminase 5 (TGM5) from *Homo sapiens*, (b) surface representation of TGM5 with residues highlighted in yellow in the binding pockets (blue)

Both the wild and mutated model of TGM5 was submitted for docking with IVL protein on ZDock server. The wild type TGM5 and IVL interacted with each other at the adjacent to the catalytic domain. Whereas in the mutated protein, no 3D docked structure indicated any proximity to catalytic site, hence it could be implied that the mutation had affected the binding of mTGM5 with IVL. The failure of docking indicates that the mutation in TGM5 (G113C) induces a change in the activity of the TGM. Structural analysis of TGM5 indicated there is a cysteine residue in the vicinity of G113, and the mutation of G113C enables the formation of cysteine bonds formation thus disrupting the secondary structure stabilization (Fig. 2c).

DISCUSSION

Molecular modeling and relative binding mechanism of TGM5, a protein performing TGM activity was done using MAESTRO and Z dock server. The protein was found to have a consistent structure as found in TGM3, TGM1, and hFX XIIIA (Human factor XIII A) comprising amino terminal β-sandwich domain; the catalytic core domain which contains the conserved active site triad Cys272, His330, and Asp353 (corresponding to TGM3 residue numbers); the β-barrel 1 domain; and the β-barrel 2 domain at the carboxy terminus. The sulfhydryl group of Cys272 forms thiolate-imidazolium ion pair with His330. His330 and Asp 353 were found to form hydrogen bond with the terminal oxygen atom of Asp353. In addition, indole rings of two tryptophan residues (Trp236 and Trp327 corresponding to TGM3) involved in the isopeptide crosslink bond formation was also found to be conserved [24,25].

Structural analysis of the TGM5 indicated that on mutation specifically G113C, there is a deviation in the basic backbone structure of the mutated protein. This is because of the presence of extra reactive sulphydryl group present in the vicinity of the active site, disturbs the packing arrangement of the native protein leading to increased dynamics within the protein. The presence of a free cysteine residue has the probability of forming unwanted intra-molecular disulfide scrambling, and covalent oligomerization via intermolecular disulfide formation [26]. The mutation (G113C) thus, affects the secondary and tertiary structure and interaction of TGM5 with IVL. It has been noticed that heat and humidity increases the symptomatic effect of TGM5 mutation [27]. With increase in temperature, the flexibility and fluidity of proteins have been found to increase [28]. Thus in addition to the extra thiol residue, moisture and increased temperature, makes the mutant TGM5 further dynamic leading to least possibility of interaction with IVL and in turn affecting isopeptide bond formation, resulting in the symptomatic effect of the mutated protein. Hence, the symptomatic effects of APSS have been observed mainly during high temperature and humidity.

CONCLUSION

Mutation G113C affects the structural rigidity of TGM5, which hinders the interface chemistry of TGM5 and IVL. Fluctuation in temperature and humidity further facilitates an increased dynamics of the TGM5, thus aggravating the symptom of APSS.

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