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Analysis of Matrix Metalloproteinases 13(MMP-13) non-synonymous Single Nucleotide Variants (nsSNVs) in Osteoarthritis and Prediction of Druggable Binding Sites Using COSMIC

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Abstract: Osteoarthritis (OA) is a degenerative joint disease characterized by progressive loss and damage of articular cartilage. Increased collagenase activity has a direct impact in the cleavage of type II collagen in osteoarthritis. Somatic mutations such as non-synonymous single nucleotide variants (nsSNVs) in coding regions have a role in the pathogenesis of a number of diseases including OA. Matrix metalloproteinase 13 (MMP-13) is a critical target gene in the progression of osteoarthritis. Liver injury and regeneration have both been linked to MMP-13 gene expression. Analysis of MMP-13 gene was conducted using online bioinformatics tool, COSMIC and nsSNVs contributing to possible accumulation events that leads to osteoarthritic phenotypes were identified. The most frequently occurring missense mutation was found to be p.T280M; threonine to methionine that showed gain in function. Moreover, to get insight into the effect of mutation on protein structure and drug binding, MMP-13 three-dimensional structure was analyzed through COSMIC 3D. Structural analyses revealed amino acid substitutions located in hemopexin (HPX) domain. Hemopexin domains are thought to be required in the activation of matrix metalloproteinases (MMPs) via increased activation of pro enzyme and thus, increased collagen hydrolysis. Therefore, there may be a possibility to modify enzyme activity through antagonist molecules targeted to such alternative enzyme domains. For this reason possible drug binding sites were also identified using COSMIC 3D. An understanding of the mechanisms of activation of procollagenases is important to prevent cartilage destruction and could be utilized for the rational design of novel MMP-13 inhibitors.

Keywords: Osteoarthritis**,** collagenase 3, matrix metalloproteinase -13, non synonymous single nucleotide variants, COSMIC

INTRODUCTION

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by progressive loss and damage of articular cartilage [1]. One of the leading cause of osteoarthritis is the elevated production and activity of proteolytic enzymes matrix metalloproteinases (MMPs) amongst which matrix metalloproteinase 13 (MMP-13) plays an important role in targeting cartilage for degradation and is more concentrated in connective tissue [2]. One of the studies demonstrated that "MMP13 is a critical target gene during the progression of osteoarthritis" [3]. Clinical investigations revealed that patients with articular cartilage destruction have high MMP13expression in chondrocytes as compared to normal knee cartilage.[4] Most MMPs including MMP13 are secreted as inactive proenzymes with an N-terminal 80-residue pro-domain. Activation and proteolytic removal of prodomain leads to MMPs activation and catalysis [5]. It was stated that hemopexin (HPX) domains of human proteins are also required in the activation of MMPs leading to efficient collagen cleavage [6]. Further studies revealed that hemopexin domains participate in the activation or/and inhibition of MMPs [7]. To date several investigations utilizing crystallographic and solution NMR techniques [8, 9] revealed that a transient short lived complex is formed between MMP hemopexin domain and triple helical collagen, which explains that any dysregulation in this regards can give rise to degradation and pathological conditions as in OA.

A significant feature of OA is the excessive production of inflammatory mediators, among which, proinflammatory cytokine interleukin-1β (IL-1) plays a crucial role in the pathophysiology along with tumor necrosis factor-α (TNF-α) and enhances matrix

metalloproteinase production. It has been demonstrated that IL-1-induced cellular activation causes agonistic increase in MMP -13 gene expression, resulting in the degradation of 3-D matrix [10, 11].

Liver injury and regeneration have both been linked to complex extracellular matrix (ECM) related pathways. MMPs have been found to be expressed in a number of acute and chronic liver disorders [12]. MMP-13 (collagenase-3) is expressed in acute liver injury and in accelerated liver fibrogenesis [13, 14].

Therefore, there is a great need to understand molecular mechanisms responsible for the development and progression of OA. Nowadays ample supply of genomic data is used to identify disease-associated genes or genomic regions [15]. Genetic alterations such as non-synonymous single nucleotide mutations play a critical role in human diseases. "Non-synonymous single nucleotide variants (nsSNVs) in coding regions of human genes frequently lead to pathological phenotypes" [16]. It is thought that genetic mutations are one of the highest risks in OA and multiple gene variations are responsible for the progression of OA [17]. Several bioinformatics tools play a pivotal role in providing databases for the detailed analysis of key mutations in genes that might be responsible for a particular disease or pathological condition [18, 19]. One such example is "Catalogue of Somatic Mutations in Cancer" (COSMIC) (http://cancer.sanger.ac.uk/cosmic) which provides online query interface for the analysis of somatic mutation frequencies in cancer genes [20].

Also one can improve understanding by visualizing these mutations three dimensionally to predict novel druggable targets. COSMIC 3D (http://cancer.sanger.ac.uk/cosmic3d/) is a best known tool in this regard which is a platform for understanding mutations in the context of three dimensional protein structure.

In the present study genetic variations in MMP-13 gene were determined using COSMIC to computationally analyze cumulative effects of nsSNVs and their impact on pathogenicity that might influence the risk and progression of OA.

As the sequence identity of 50%–60%, MMP catalytic domain structures are highly similar, this novel approach is highly advantageous in determining binding sites other than the catalytic sites as targets for the potential allosteric control of MMP domains.

MATERIALS AND METHODS

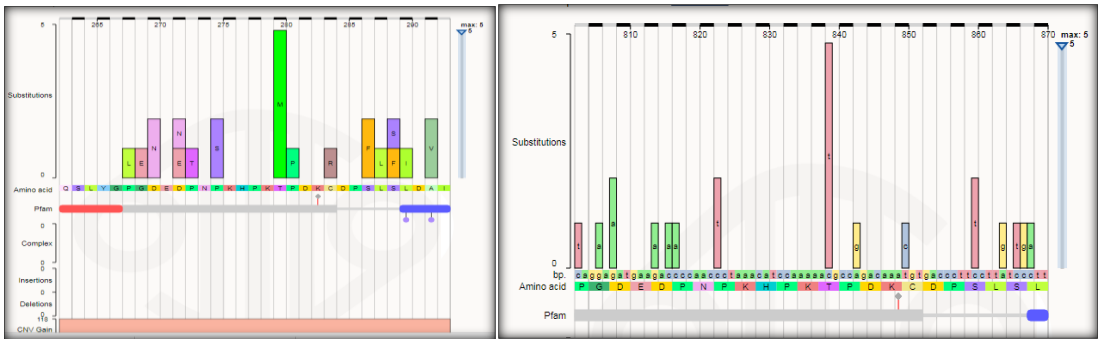
MMP-13 mutation data was analyzed through online bioinformatics tool, Catalogue of Somatic Mutations in Cancer (COSMIC) that could be retrieved from (http://cancer.sanger.ac.uk/cosmic). Three dimensional visualization and analysis of key MMP-13 mutations were carried out through COSMIC 3D (http://cancer.sanger.ac.uk/cosmic3d). Crystal structures of both full form of Collagenase 3 (MMP-13) with attached prodomain peptides and C terminal hemopexin-like domain were chosen for the detailed analysis of nsSNVs with pdb Ids 4G0D and 1PEX respectively.

Predicted Small-Molecule Druggable Binding sites were generated through COSMIC 3D and the amino acids involved in interactions with small molecules were also identified.

RESULTS

The most frequent amino acid mutation was found to be p.T280 M (c.839 C>T) threonine to methionine i.e. from polar to non polar amino acid at residue position 280 and showed gain in function. Histogram representing most frequent MMP-13 gene mutation on amino acid scale and nucleotide scale is shown in Figure 1a and 1b respectively.

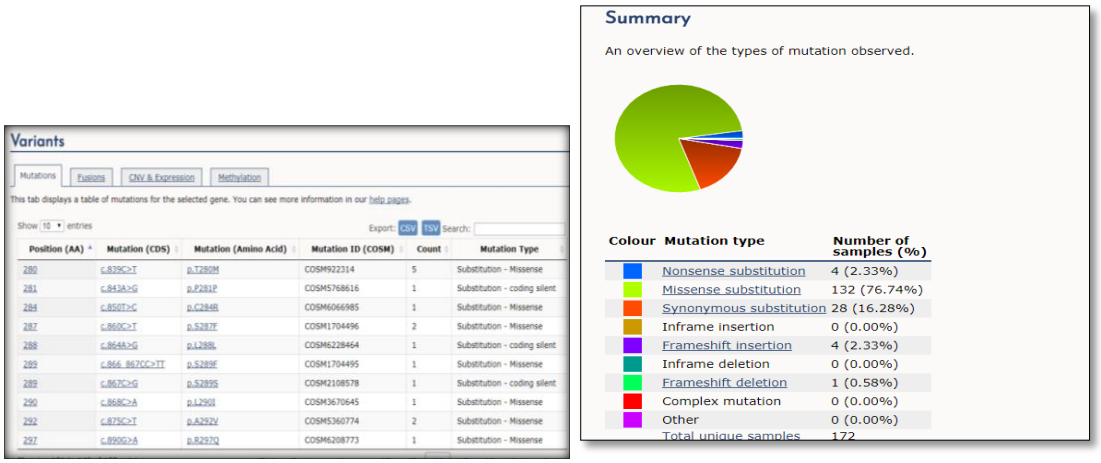
MMP-13 gene analysis showed that codon 280 encodes the key threonine residue which might be frequently mutated in osteoarthritis to methionine particularly nucleotides g838 and g839.



a **b**

Fig-1: Graphical representation of the mutation spectrum across the MMP-13 gene on the amino acid scale (a) and on the nucleotide scale (b)

Summary of some of the complex mutations and the percentage of its different types is given in figure **2a** and **2b** observed in 172 samples.

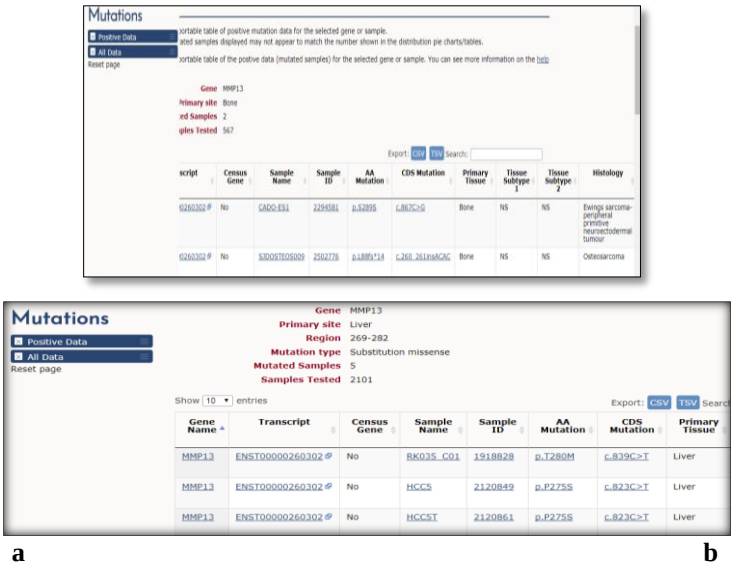


a **b**

Fig-2: Novel introduction of complex mutations in MMP-13; the most frequent one is p.T280 M (c.839 C>T) on the top (a) Summary of percentage of different types of mutations observed in 172 samples MMP-13 (b)

Tissue specific mutations analyzed for *bone* and *liver* are shown figure **3a** and **3b** respectively. Analysis of bone as a primary tissue showed insertion at position 88 in amino acid region (p.L88fs) and silent mutation (p.S289S) with substitution c.867 C>G that usually does not affect phenotype.

Therefore, the study was further elaborated in liver to look for key missense substitutions in liver that could possibly have indirect effect on MMP-13 gene expression and activation. Again pT280 M (c.839 C>T) was found to be a major missense substitution in liver in addition with some other frequently found mutations i.e. p.P275S (Phenylalanine to Serine at nucleotide g823) (c.823 C>T) in HPX domain.



a **b**

Fig-3: Tissue specific mutations in (a) bone and (b) liver

In bone, out of 567 samples only 2 mutated samples were found, whereas in liver, the number of over expressed samples were 15 out of 373 showing total percentage of 4.02. Histogram of key mutations observed in bone and liver are shown in figures **4a** and **4b**.

a **b**

Fig-4: Tissue-specific mutation spectra in the MMP-13 gene for (a) bone and (b) liver

This showed that these mutations are causing non synonymous change in the sequence of amino acids i.e. from polar to nonpolar that greatly affects the overall structural and functional characteristic of protein resulting in enhanced MMp-13 pro enzyme activation.

Figure **5a** and **5b** is showing three dimensional visualization of mutations (p.T280 M near hemopexin domain) with pdb Ids 4G0D and 1PEX respectively.

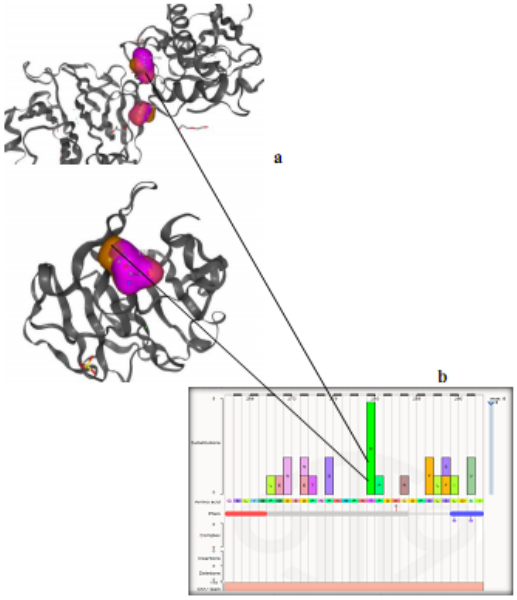


Fig-5: Three dimensional structure of MMP-13 with pdb Id 4G0D (a) and 1PEX (b) showing p.T280 M mutation near hemopexin domain. The wild type T280 is shown in pink whereas mutant M280 is modeled in orange

Two druggable small molecule binding sites (Table 1&2) were identified in MMP-13(pdb Id 1PEX) generated by COSMIC 3D with the druggability score of 0.628 and 0.636 respectively.

Table 1: Druggability of predicted binding site

Property	**Value**
Druggability Score	0.636
Volume	118 $Å^3$
Density Proxy for buriedness	3.3

Table 2: Druggability of predicted binding site

Property	**Value**
Druggability Score	0.628
Volume	615 $Å^3$
Density Proxy for buriedness	6.5

Three dimensional visualization of two binding sites in HPX domain is shown in figure **6**.

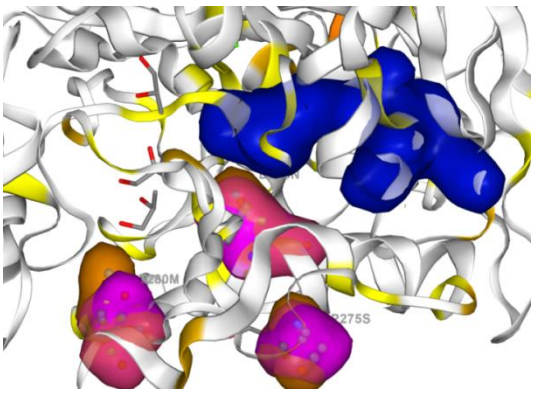
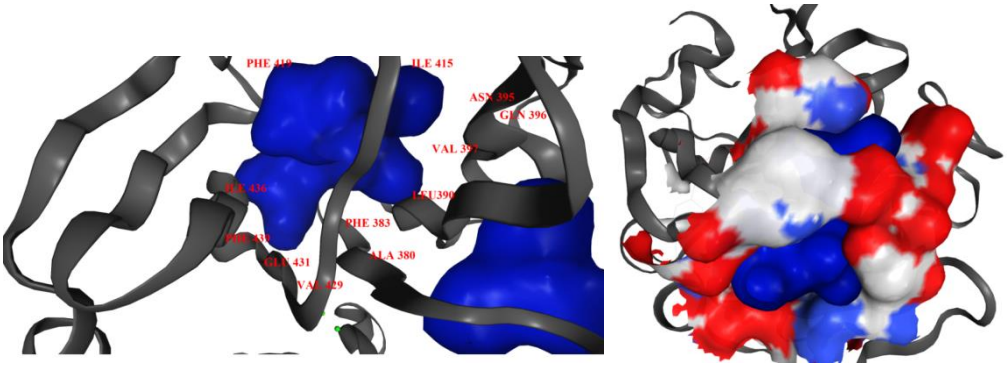


Fig-6: Predicted druggable small molecule binding pockets seen in blue color whereas mutations are seen in orange color

Binding pocket with labeled hydrophobic amino acids is given in figure **7a**. Analysis of binding pocket revealed the presence of hydrophobic amino acids such as Ala 380, Leu 390, Phe 383 and 490, Val 397, Ile 415 etc. in HPX domain that might be created as a result of p.T280 M mutation. Therefore, elucidating the structural modifications of the HPX domain is significant to fully understand the process of MMP-13 over expression and activation and may be utilized as therapeutic target for rational drug designing.



a **b**

Fig-7: Hydrophobic pocket with visible hydrophobic amino acid residues (a) Solvent-accessible surface showing negatively charged hydrophobic cavity in red (b)

Solvent accessible surface with hydrophobic pocket is represented in figure **7b**. The pocket is colored based on Eisenberg's hydrophobicity scale. Basically, the deeper the red, the more hydrophobic the residue which is clearly visible in the figure.

CONCLUSION

Despite tremendous efforts over the past decade, only a few selective and effective drugs with desired properties have emerged for inhibiting individual MMPs and have been shown to be dependent on domains other than the catalytic domain.

This novel approach highlighted the non-synonymous single nucleotide variants (nsSNVs) in coding regions and the role of hemopexin domain in the over expression and activation of MMP-13 which may open doors for the designing of selective inhibitors.

It was also concluded that acute and chronic liver disorders might contribute in MMp-13 gene expression.

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