PHYLOGENETIC TREE CONSTRUCTION FOR HIGHLY CONSERVED MITOGEN ACTIVATED PROTEIN KINASES

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ABSTRACT - Mitogen activated protein kinases (MAPKs) are stimulated by a large variety of signals, including mitogens, growth factors, cytokines, T-cell antigens, pheromones, UV and ionizing radiations, osmotic stress, heat shock and oxidative stress. They participate in the generation of various cellular responses, including gene transcription, induction of cell death or maintenance of cell survival, malignant transformation, and regulation of cell-cycle progression. MAPKs are involved in the action of most nonnuclear oncogenes and responsible for cell response to growth factors. MAPK pathway has been shown to play a pivotal role in diverse dental diseases, including chronic pain, and periodontal diseases. In this work, an attempt has been made to determine the participation of particular MAPK in one specific pathway. Various computational analysis tools such as Clustal W, phylogenetic tree re-construction, PDB, Phosphosite etc were utilized and based on evolutionary relationships, identification of phosphorylated sites and comparison of active site residues, the specificity of MAPK 1 and 3 in growth factor pathway, MAPK 8,9,10 in stress and MAPK 11,12,13 in inflammatory pathway are emphasized.

Keywords: *MAPK, Pathway, Phosphorylation, active site residues*

I. INTRODUCTION

The Mitogen-activated protein kinase (MAPK) family belongs to the eukaryotic protein kinase super family. MAP kinases were identified by virtue of their activation in response to growth factor stimulation of cells hence the name mitogen activated protein kinases [1-3]. The transmission of extracellular signals into their intracellular targets is mediated by a network of interacting proteins that regulate a large number of cellular processes. Cumulative efforts from many laboratories over the past decade have allowed the elucidation of one such signaling mechanism, which involves activations of several membranal signaling molecules followed by a sequential stimulation of several cytoplasmic protein kinases collectively known as mitogen- activated protein kinase (MAPK) signaling cascade [4-5]. Another physiological response that appears to be regulated through the MAPK signaling pathway is cellular differentiation. Different members of the MAPK cascade have been implicated in processes such as monocytic differentiation, neunte outgrowth of PC12 cells, T cell maturation, and mast cell development. The activity of most MAPK's is stimulated by a large variety of signals, including mitogens, growth factors, cytokines, T cell antigens, pheromones, phorbol esters, UV and ionizing radation, osmotic stress etc [6-7].

Mitogen-activated protein (Map) kinases are widely expressed serine-threonine kinases that mediate important regulatory signals in the cell. Three major groups of Map kinases exist: the p38 Map kinase family, the extracellular signal-regulated kinase (Erk) family, and the c-Jun NH_2-terminal kinase (JNK) family. The members of the different Map kinase groups participate in the generation of various cellular responses, including gene transcription, induction of cell death or maintenance of cell survival, malignant transformation, and regulation of cell-cycle progression. Extracellular information perceived at the surface of a cell must be translated into an intracellular response that involves a complex network of interwoven signalling cascades. These signalling events ultimately regulate cellular responses such as proliferation, differentiation, secretion and apoptosis [8-10]. In general phosphorylation either activates or inactivates a given protein to perform a certain function. Protein kinases and phosphotases are responsible for determining the phosphorylation state of cellular proteins in the subcellular localization and activity of kinases and phosphatases have consequences for normal cell function and maintenance of cellular homeostasis [11]. Over the last few years, extensive work by several groups has established that Map kinase pathways play critical roles in the pathogenesis of various hematologic malignancies, providing new molecular targets for future therapeutic approaches [12].

The rationale of this work is to study multiple sequence analysis of MAPK involved in various pathways in order to ascertain the distribution of specific MAP Kinase pathways. In other words to determine the MAPK protein specificity and participation in definite pathway by exploring the structural differences in terms of conserved and variant residues within active site regions of MAPKs was presented.

II. MATERIALS AND METHODS

MAP Kinase proteins involved in growth factor (Ras activation), stress (TGF activation) and inflammatory related pathways (fig 1) are taken from Biocarta. All protein sequences of MAPKs are extracted from ExPASy server. ClustalW multiple sequence alignment program was used to produce biologically meaningful multiple sequence alignment of divergent sequences.

Phylogenetic relationships among MAPK proteins (Figure 1) were carried out by using ClustalW alignment program. Proteins in fasta format are downloaded from swissprot protein sequence database and they are subjected to multiple alignment in clustalw with default parameters. The resulted alignment and trees are studied to evaluate evolutionary relationships and divergences among sequences.

III. RESULTS AND DISCUSSION

The ever evolving mitogen-activated protein kinase (MAP kinase) pathways consist of four major groupings and numerous related proteins which constitute interrelated signal transduction cascades activated by stimuli such as growth factors, stress, cytokines and inflammation. The four major groupings are the Erk (red), JNK or SAPK (blue), p38 (green) and the Big MAPK or ERK5 (light blue) cascades [13, 14]. The core unit of mitogen-activated protein kinase (MAPK) pathways is a three member protein kinase cascade. Within the three-kinase module, MAPKs are phosphorylated and activated by MAPK kinases (MKKs). The MKKs are themselves phosphorylated and activated by serine/threonine kinases. Activation of MAPKs in response to these stimuli controls gene expression, metabolism, cytoskeletal functions and other cellular regulatory events. The sequences of MAPK 1 to 13 which participate in the growth factor, stress, inflammatory pathway are analyzed and the phylogenetic tree was obtained (figure 2).

3.1 Phylogeny

From the phylogenetic tree, it is evidenced that all MAPKinases 1 to 13 in humans participate in different pathways. MAPK1 and 7 are on one taxon and role of MAPK7 is unknown but MAPK1 is involved in GROWTH FACTOR PATHWAY. MAPK 2, 3 and 5 are on one taxon. MAPK3 is involved in GROWTH FACTOR PATHWAY and remaining two MAPKinases does not participate in any of the signalling pathways of the MAPK's. MAPK4 and 6 is on one taxon. These two kinases is also does not participate in any of the signalling pathways. MAPK8,9 and 10 are on one taxon . These three are involved in STRESS PATHWAY. MAPK11, 12 and 13 are on one taxon and these three are involved in INFLAMMATORY PATHWAY.

3.2 Identification of Phosphorylation Sites

3.2.1 Growth Factor Pathway

Presented in tables:

All MAPKs are investigated for the presence of similarities of specific amino acid residues at the active site regions. Not all MAPKs have 3-dimensional structure and hence comparison of active site residues were made with known 3-D strctures using multiple alignments. Table 9 presents the information about MAP Kinases participating in stress, growth and inflammatory pathways and its PDB structures.

The present work reported the identification of pathway specificity of MAPK's (participating in the MAPK signaling pathway. The sequence of all the MAPK's which participate in the pathway are extracted from EXPASY (Expert protein analysis system). Around 13 sequences of humans are obtained and are subjected to Sequence Analysis and Phylogenetic Analysis by using CLUSTALW. It has been observed from the analyses that the path specific MAPK's share a very close proximity on the phylogenetic trees by originating from a common node and diverging to a very small extent. Finally the path specificity of the MAPK sequences is confirmed by applying the results of the above analyses for better understanding.

Figures and Tables

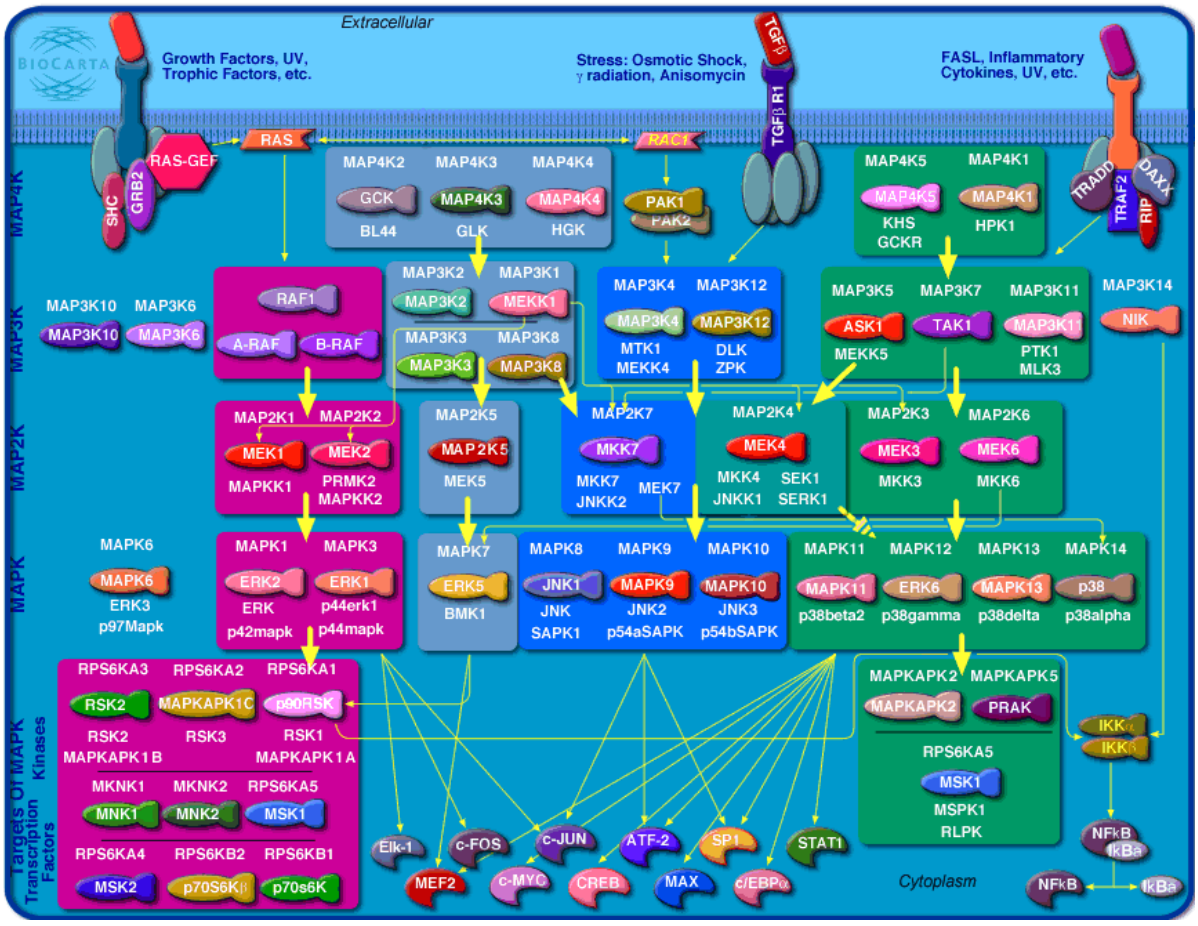

FIGURE 1: MAPKinase signalling pathway

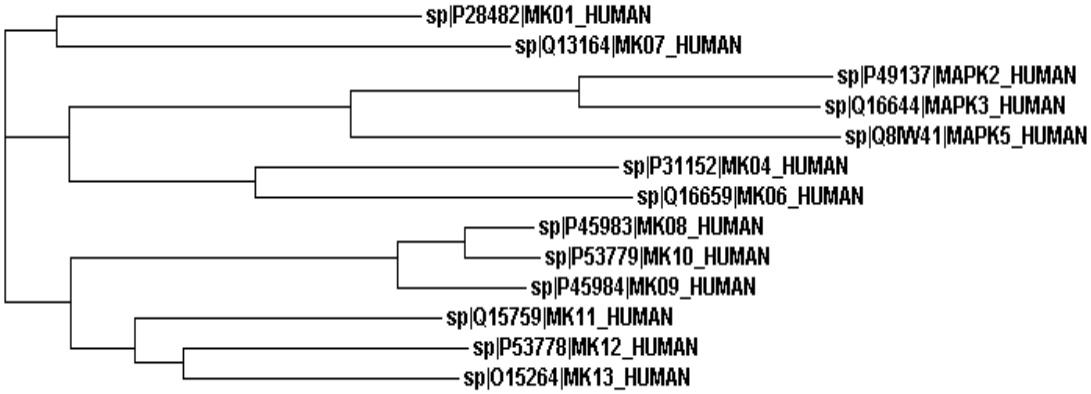

FIGURE 2: Phylogenetic tree of MAPK's.

MAPK1:

Table 1: Phosphorylation sites of MAPK1.

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PDA1T9W2	11	30	SERINE-28
PD972663	19	100	THREONINE-62
PD000001	31	230	THREONINE180,184 ,189 TYROSINE186 SERINE-201
PD696917	277	312	SERINE-283

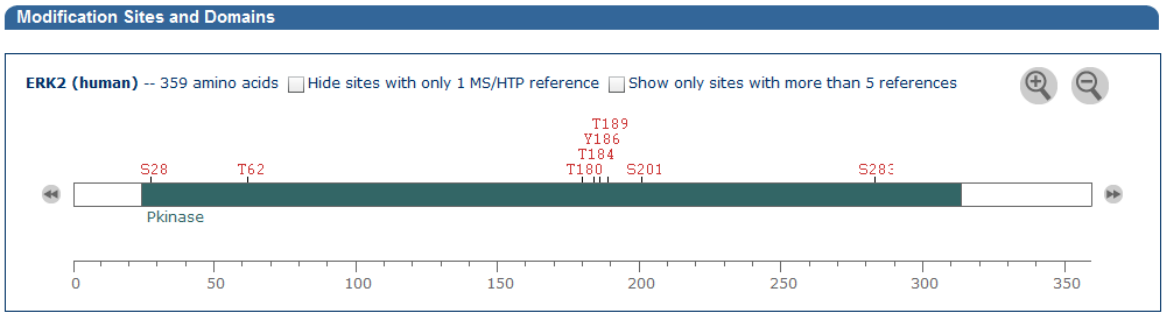

Figure 4: Phosphorylation sites in MAPK1 on all domains.

MAPK3:

Table 2: Phosphorylation sites of MAPK3.

DOMAINS	BEGINING	ENDING	PHOSPHORYLATION SITES
PD000001	43	303	THREONINE-201 TYROSINE-204,207,208 SERINE251
PDA1I9T1	304	332	THREONINE-313,317

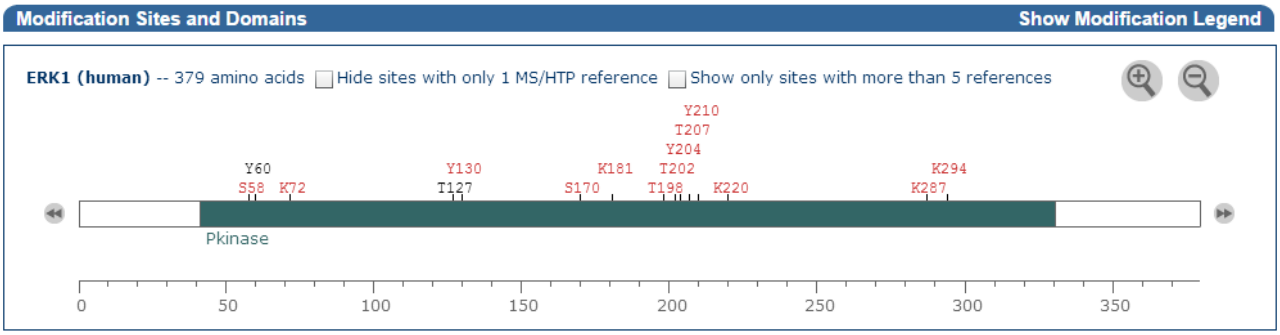

Figure 5: Phosphorylation sites in MAPK3 on all domains

3.2.2 Stress Related Pathway

MAPK8:

Table 3: Phosphorylation sites of MAPK8

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PD067788	10	171	SERINE-155
PD000001	29	274	THERONINE-178,183,188 TYROSINE-185

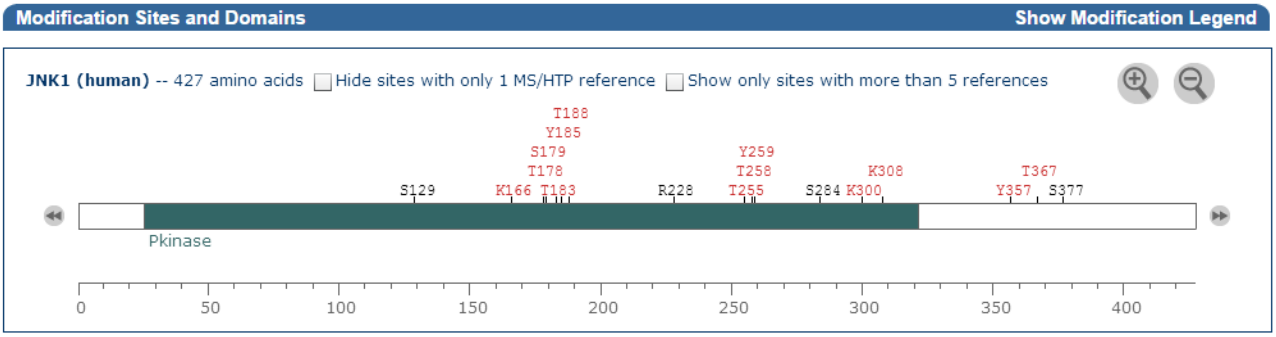

Figure 6: Phosphorylation sites in MAPK8 on all domains.

MAPK9

Table 4: Phosphorylation sites of MAPK9

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PD067788	8	227	SERINE-155 THERONINE-183 TYROSINE-185

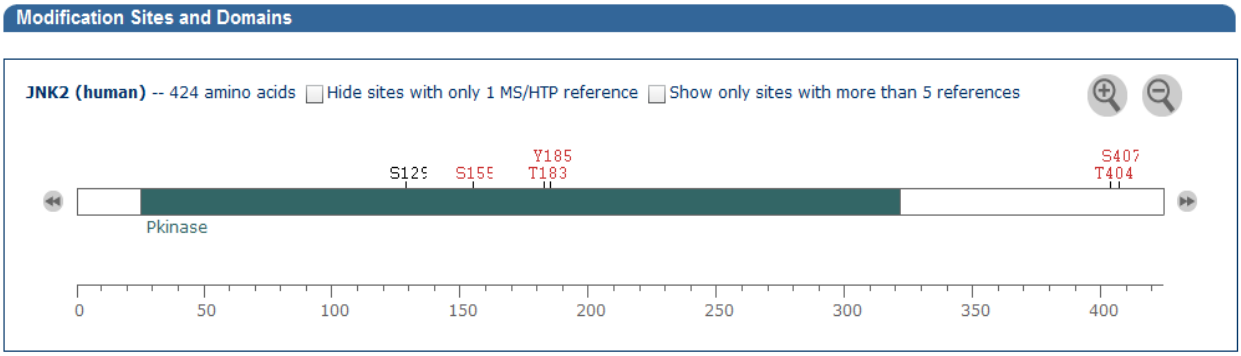

Figure 7: Phosphorylation sites in MAPK9 on PD067788 domain.

MAPK10

Table 5: Phosphorylation sites of MAPK10

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PD067788	48	209	SERINE-193

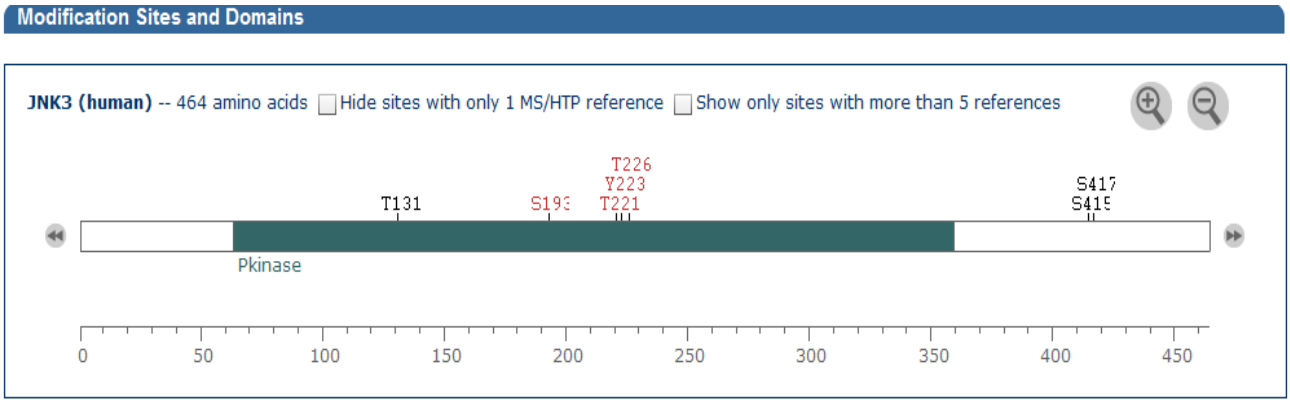

Figure 8: Phosphorylation sites in MAPK10 on PD067788 domain.

3.2.3 INFLAMMATORY PATHWAY

MAPK11:

Table 6: Phosphorylation sites of MAPK11

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PD067788	20	209	TYROSINE-182
PD000001	27	243	SERINE-243

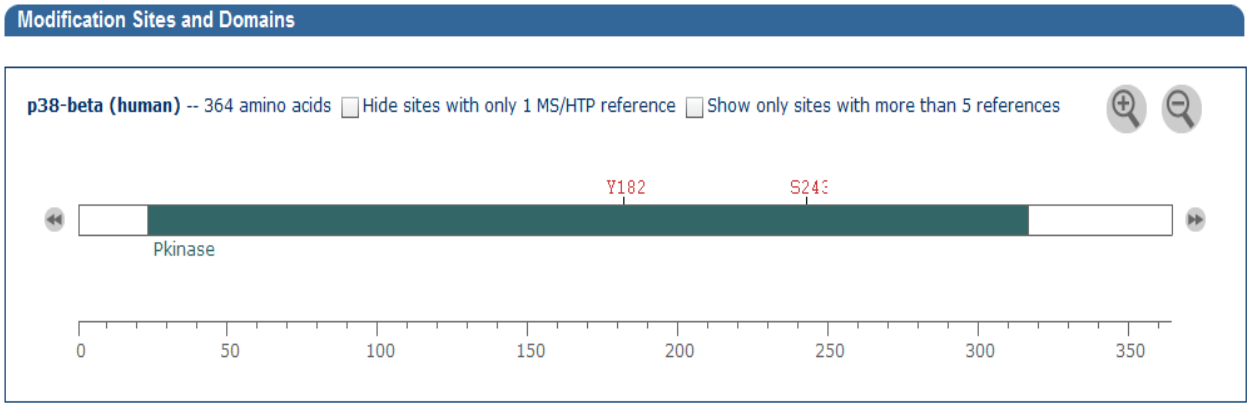

Figure 9: Phosphorylation sites in MAPK11 on PD067788 and PD000001 domains.

MAPK12:

Table 7: Phosphorylation sites of MAPK12

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PDA1T9N7	1	26	SERINE-3
PD000001	27	265	THERONINE-183 TYROSINE-185

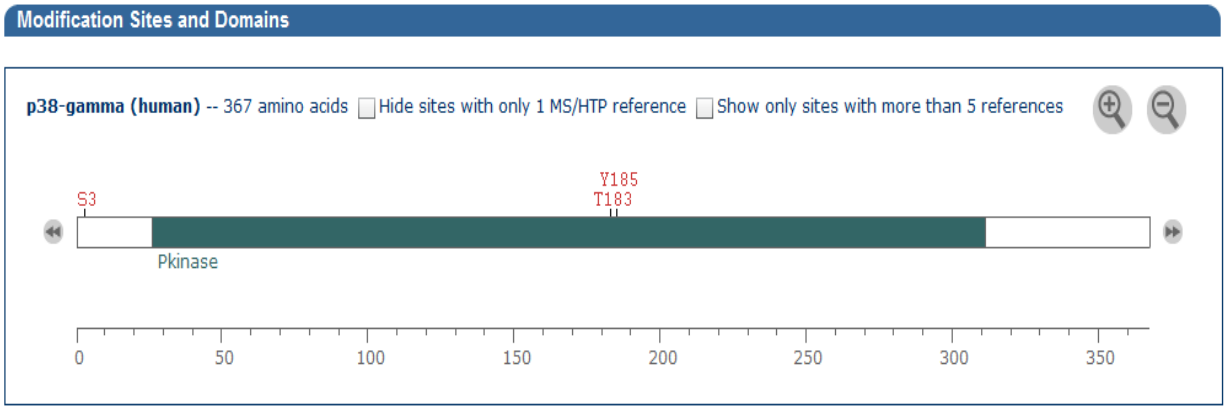

Figure 10: Phosphorylation sites in MAPK12 on PDA1T9N7 and PD000001 domains.

MAPK13:

Table 8: Phosphorylation sites of MAPK13

DOMAINS	BEGINING	ENDING	PHOSPHORYLATED SITES
PD000001	32	237	SERINE-47 TYROSINE-182

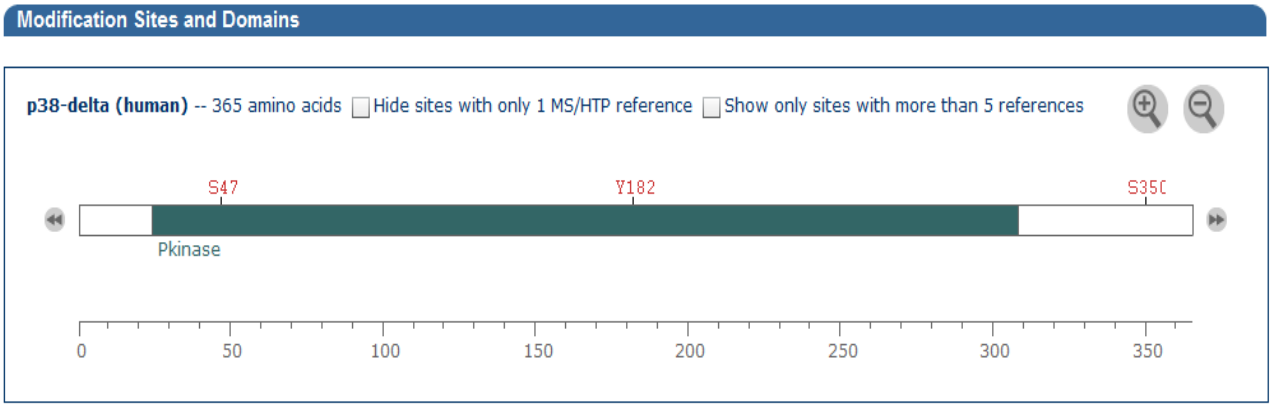

Figure 11: Phosphorylation sites in MAPK13 on PD000001 domain.

Table 9: MAPK kinases with their uniprot id and PDB entries.

S No	MAPKINASES	Uniprot ID	PATHWAY	PDB SRRUCTURE
1	MAPK 1	P28482	GROWTH	1TVO
2	MAPK 2	P49137	UNKNOWN	UN DETERMINED
3	MAPK 3	Q16644	GROWTH	2Z0Q
4	MAPK 4	P31152	UNKNOWN	UN DETERMINED
5	MAPK 5	Q8IW41	UNKNOWN	UN DETERMINED
6	MAPK 6	Q16659	UNKNOWN	216L
7	MAPK 7	Q13164	UNKNOWN	UN DETERMINED
8	MAPK 8	P45983	STRESS	1UKI
9	MAPK 9	P45984	STRESS	3E70
10	MAPK 10	P53779	STRESS	1JNK
11	MAPK 11	Q15759	INFLAMMATORY	3GCB
12	MAPK 12	P53778	INFLAMMATORY	1CM8
13	MAPK 13	015264	INFLAMMATORY	3C01

14	MAP2K1	Q02750	GROWTH	1S9J
15	MAP2K2	P36507	GROWTH	1S9
16	MAP2K3	P46734	INFLAMMATORY	UN DETERMINED
17	MAP2K4	P45985	STRESS	UN DETERMINED
18	MAP2K5	Q13163	UNKNOWN	2NPT
19	MAP2K6	P52564	INFLAMMATORY	3ENM
20	MAP2K7	014733	STRESS	2DYL
21	MAP3K1	Q13233	UNKNOWN	UN DETERMINED
22	MAP3K2	Q9Y2U5	UNKNOWN	2NPT
23	MAP3K4	Q9Y6R4	STRESS	UN DETERMINED
24	MAP3K5	Q99683	INFLAMMATORY	
25	MAP3K7	043318	INFLAMMATORY	2EVA
26	MAP3K11	Q16584	INFLAMMATORY	UN DETERMINED
27	MAP3K12	Q12852	STRESS	
28	MAP4K1	Q92918	INFLAMMATORY	UN DETERMINED
29	MAP4K2	Q12851	UNKNOWN	UN DETERMINED
30	MAP4K3	Q8IVH8	UNKNWON	UN DETERMINED
31	MAP4K4	095819	UNKNWON	UN DETERMINED
32	MAP4K5	Q9Y4K4	INFLAMMATORY	UN DETERMINED

IV. CONCLUSION

The specificity of the MAPK's to a particular pathway was studied from the Phylogenetic Tree Analysis and Identification of phosphorylated sites, Active site amino acids. Computational analysis provided identification of specific MAPK participating in a particular pathway and phylogenetic studies revealed that the path specific MAPK's show close proximity in the phylogenetic tree, originating from a common node and diverging to a very extent, thus showing greater degree of similarity among them. Phosphorylated sites revealed similar and dissimilar domains in MAPK's. Amino acid variations among MAPKs suggest that specificity in protein sequence conservation exists among all MAP kinases and those with slight variations participate in defined pathways.

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