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In-Silico Molecular Characterization of MST1 Protein

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ABSTRACT

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes cases up to 422 million worldwide; India ranks among top 3 countries with diabetic population. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Two distinct mechanisms are responsible for the death of β cells one through ER stress induced death and the other being cytokine mediated death via apoptosis. Various literature and the molecular pathways emphasizes the involvement of one major protein for β cell death named as MST-1. Mammalian Sterile-20-like kinase (MST1, also known as STK4, KRS2) is an ubiquitously expressed serine/threonine kinase, it is part of the Hippo signaling pathway and involved in multiple cellular processes such as morphogenesis, proliferation, stress response and apoptosis. Hence our present investigations focus mainly to understand the detailed molecular features of MST-1 by retrieving its protein sequence from major protein sequence repository, analysing the physico-chemical properties, secondary structural elements and tertiary structure prediction using various databases, tools and techniques of bioinformatics.

Keywords : Diabetes Mellitus, β Cell Death, STK4, KRS2, MST1 Protein.

I. INTRODUCTION

Diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. Diabetes cases up to 422 million worldwide; India ranks among top 3 countries with diabetic population. Ahead of World Health Day (April 7), the numbers climbed from 20.4 million in China in 1980 to 102.9 million in 2014, the rise has been equally dramatic in India from 11.9 million in 1980 to 64.5 million in India. [Sarwar N (2010)] .Type 1 DM results from the pancreas's failure to produce enough insulin.

This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown. [*Chiang (2014)]* Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly.
MST1 a protein kinase of the STE20 family. Proteolytically activated by caspase during apoptosis. Activated by apoptotic signals as well as other stress conditions. Full activation requires both phosphorylation and caspase-mediated cleavage. Phosphorylation at serine 327 of Mst1, which is close to the caspase-3 recognition site, inhibits caspase-mediated cleavage.

Mammalian sterile 20–like kinase-1 (MST1) as a critical regulator of apoptotic beta cell death and function. Under diabetogenic conditions, MST1 was strongly activated in beta cells in human and mouse islets and specifically induced the mitochondrial-dependent pathway of apoptosis through upregulation of the BCL-2 homology-3 (BH3)-only protein BIM. MST1 directly phosphorylated the beta cell

transcription factor PDX1 at T11, resulting in the latter's ubiquitination and degradation and thus in impaired insulin secretion. MST1 deficiency completely restored normoglycemia, beta cell function. [Taylor LK (1996)]

II. METHODS AND MATERIAL

A. Retrieval of protein sequence information of MST1

For the study of MST1 molecule, its amino acid sequence was retrieved from the major protein sequence databases like UniprotKB and NCBI protein database. The sequence obtained was stored in Fasta format with its accession number [Barker (2001)]

B. Analysis of Physicochemical properties

The analysis of physicochemical properties of MST1as done by using protein prediction tool Protparam which gives a detailed information of protein like isoelectrical point, theoretical pI extension coefficient, half life, instability index, Aliphatic index, Grand average, hydropathicity, etc. [William L (2009)]

C. Secondary structure prediction of MST1

The secondary structure prediction of MST1 was carried out by using online secondary structure prediction tool SOPMA, which gives the information of Alpha helix, beta sheets, extended strands and random coils. [Pirovano (2010)]

D. Analysis of catalytic site of MST1

The analysis of catalytic site of MST1 protein was performed by using Catalytic Site Atlas. The possible number of catalytic sites with the residues involved were highlighted for each site. [Skeel A (1991)]

E. Prediction of Tertiary structure of MST1

The Tertiary structure of MST1 was obtained by using SWISS Model tool by selecting the template with maximum homology and with optimised parameters. The obtained structure was stored in pdb format for visualization.

F. Visualization of tertiary structure of MST1

The predicted Tertiary structure of MST1 was visualized by using structure visualization tool Rasmol. Visualization was done using different models and formats to understand structural features of MST1. [Waltz SE (1996)]

III. RESULTS AND DISCUSSION

A. Retrieval of protein sequence information of MST1

For the study of MST1 molecule, its amino acid sequence was retrieved from the major protein sequence databases like UniprotKB and NCBI protein database. The sequence obtained was stored in Fasta format with its accession number. The length of the sequence was found to be 487 aa as shown in figure 1.

Accession no.- Q13043

STK4_HUMAN Serine/threonine-protein kinase 4 OS=Homo sapiens GN=STK4 PE=1 SV=2
>METVQLRNPPRRQLKKLDEDSLTKQPEEVFDVL
EKLGEGSYGSVYKAIHKETGQIVAIKQVPVESDLQ
EIIKEISIMQQCDSPHVVKYYGSYFKNTDLWIVME
YCGAGSVSDIIRLRNKTLTEDEIATILQSTLKGLEY
LHFMRKIHRDIKAGNILLNTEGHAKLADFGVAGQ
LTDTMAKRNTVIGTPFWMAPEVIQEIGYNCVADI
WSLGITAIEMAEGKPPYADIHPMRAIFMIPTNPPPT
FRKPELWSDNFTDFVKQCLVKSPEQRATATQLLQ
HPFVRSAKGVSILRDLINEAMDVKLKRQESQQRE
VDQDDEENSEEDEMDSGTMVRAVGDEMGTVRV
ASTMTDGANTMIEHDDTLPSQLGTMVINAEDEEE
EGTMKRRDETMQPAKPSFLEYFEQKEKENQINSF
GKSVPGPLKNSSDWKIPQDGDYEFLKSWTVEDLQ
KRLLALDPMMEQEIEEIRQKYQSKRQPILDAIEAK
KRRQQNF

Figure 1: Showing Protein sequence of human MST1

3.2 Analysis of Physicochemical properties

The analysis of physicochemical properties of MST1 was done by using protein prediction tool i.e. protparam which gives a detailed information of protein i.e. isoelectrical point, theoretical pI, extension coefficient, half life, instability index, Aliphatic index, Grand average, hydropathicity, etc., were predicted, calculated and tabulated as shown in table 1

TABLE 1.

Showing Physicochemical properties of normal MST1

Properties	Values
No. of amino acid	487
Molecular weight	55630.16
Theoretical pI	4.97
Total no. of negative charged residue (Asp+Glu)	82
Total no .of positively charged residue(Arg +Lys)	60
Atomic composition	
1)Carbon(C)	2443
2)Hydrogen(H)	3885
3)Nitrogen(N)	665
4)Oxygen(O)	768
5)Sulphur(S)	24
Total no .of atoms	7785
Extinction coefficient No.	51130
Half life	30hrs
Instability index	Unstable(54.85)
Aliphatic index	78.28

3.3Secondary structure predictionofMST1

Secondary structure prediction of MST1 was carried out by using SOPMA and all the secondary structural elements like alpha helix, beta sheets, random coils and extended strands were predicted as shown in table 2. [Gobel (1994)]

TABLE Π

Showing secondary structure information of MST1

Structural component	Residues	Percentage
Alpha helix (Hh)	241	49.49%
310 helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strand(Ee)	55	11.29%
Beta turn (Tt)	35	7.29%
Bend region(Ss)	0	0.00%
Random coil (Cc)	156	32.03%
Ambiguous	0	0.0%

3.4 Analysis of catalytic site of MST1

The analysis of catalytic site of MST1 protein was performed by using Catalytic Site Atlas. The possible number of catalytic sites with the residues involved were highlighted for each site as shown in figure 2.

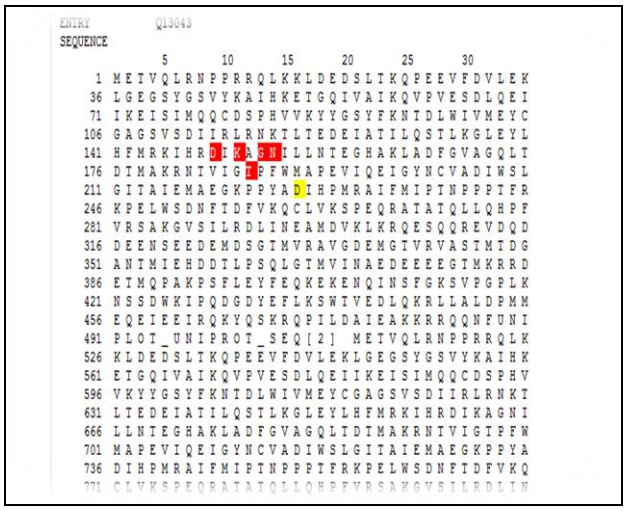

Figure 2: catalytic active site of MST 1

3.5 Prediction Tertiary structure of MST1

The Tertiary structure of MST1 was obtained by using SWISS Model tool by selecting the template with maximum homology and with optimised parameters.

The obtained structure was stored in pdb format for visualization. The details of template selected for structure prediction was as shown on table 3.

TABLE III

Showing template information of MST1 for homology modelling

Name	Title	Identity	Oligostate
4lgd.2.A	Serine/threonine-protein kinase 3	87.53	Hetero-oligomer
4lgd.3.A	Serine/threonine-protein kinase 3	87.53	Hetero-oligomer
4lgd.4.A	Serine/threonine-protein kinase 3	87.53	Herero-oligomer
4lgd.1.A	Serine/threonine-protein kinase 3	87.53	Hetero-oligomer
3com.1.A	Serine/threonine-protein kinase 4	100.00	Monomer
3com.2.A	Serine/threonine-protein kinase 4	100.00	Monomer

3.6 Visualization of tertiary structure of MST1

The predicted Tertiary structure of MST1 was visualized by using structure visualization tool Rasmol. Visualization was done using different models and formats to understand structural features of MST1 The various models were represented as shown in figure 3 . [Gettins PG (2002)]

Figure 3: Visualization of 3D structure of MST1

IV.CONCLUSION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes cases up to 422 million worldwide; India ranks among top 3 countries with diabetic population. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. Two distinct mechanisms are responsible for the death of β cells one through ER stress induced death and the other being cytokine mediated death via apoptosis. Various literature and the molecular pathways emphasizes the involvement of one major protein for β cell death named as MST-1. Mammalian Sterile-20-like kinase (MST1, also known as STK4, KRS2) is an ubiquitously expressed serine/threonine kinase, it is part of the Hippo signaling pathway and involved in multiple cellular processes such as morphogenesis, proliferation, stress response and apoptosis. Hence our present investigations focus mainly to understand the detailed molecular features of MST-1 using various databases, tools and techniques of bioinformatics.

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