

IGF2BP1: a novel binding protein of p38 MAPK

Jacob Rini¹ · Moorthy Anbalagan¹

Received: 19 February 2017 / Accepted: 4 May 2017 / Published online: 11 May 2017
© Springer Science+Business Media New York 2017

Abstract Signal transduction pathways control various biological processes in cells leading to distinct cellular functions. Protein–protein interactions and post-translational modifications are the physiological events that occur in signaling pathway. p38 MAPK are known to be involved in regulating wide range of cellular processes by interacting and activating relevant signaling molecules by means of phosphorylation. Deregulation of p38 MAPK is associated with various pathological conditions. In order to get an insight into the role played by p38 MAPK in cellular signaling, studies were carried out to identify proteins that interact with p38 MAPK. Mass spectrometry was used to identify the proteins present in p38 MAPK complex obtained by co-immunoprecipitation. Based on mass spectrometry data, here we report insulin-like growth factor-II binding protein 1 (IGF2BP1) as a novel interacting partner of p38 MAPK. IGF2BP1 is a RNA-binding protein predominantly known to be involved in tumor progression. To reconfirm the mass spectrometry data, in silico analysis was carried out. Based on different models predicted in silico, we report the possible interaction domains of p38MAPK and IGF2BP1. Considering the involvement of p38MAPK and IGF2BP1 in cancer, our study opens up the possibility of p38MAPK regulating IGF2BP1 function, and the possibility of targeting this novel interaction for developing cancer-treating drugs is discussed.

Keywords Protein–protein interactions · p38 MAPK · IGF2BP1 · Mass spectrometry · Molecular docking

✉ Moorthy Anbalagan
anbalagan.m@vit.ac.in

¹ School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu 632014, India

Introduction

In response to extracellular stimulus, various signaling cascades mediate changes in gene expression that are regulated at different levels including, post-transcriptional level. The post-transcriptional regulation involves various processes such as RNA export, stability, localization, and rate of translation which are mediated by proteins collectively known as RNA-binding proteins [1]. The involvement of RNA-binding proteins in cancer progression and metastasis has been extensively studied [2]. Among the various RNA-binding proteins, insulin-like growth factor-II binding proteins (IGF2BP) have been known to be largely involved in tumor progression.

The IGF2BP belongs to a group of conserved RNA-binding proteins that are known to regulate stability of RNA at post-transcriptional level. They are assigned numerous synonyms such as the IMP, VICKZ, ZBP KOC based on their diverse biological roles identified independently by different research groups [3–5]. IGF2BP1 is an oncofetal protein that is predominantly expressed in the embryonic tissues and cancer cells [6]. In the recent past, an upregulated expression of IGF2BP1 has been observed in various cancers including lung, ovarian, colon, brain, breast, and skin [7–9]. This protein stabilizes the c-Myc mRNA, preventing its degradation by binding to a specific sequence known as the coding region determinant (CRD) and thereby increasing c-Myc protein levels leading to cell proliferation, one of the hallmarks of cancer [10]. They are also known to regulate the translation of IGFII mRNA by binding to its 5' UTR, another factor for increasing cell proliferation [11]. As proposed by Bell et al., these proteins are termed as the “post transcriptional drivers of tumor progression” owing to their role in cancer metastasis. They are also known to positively regulate the expression of

various oncogenic factors such as KRAS and c-MYC which has a role in proliferation and MDR1 which plays a role in drug resistance of tumor cells [12]. IGF2BP1 is also known to have a role in signal transduction pathways such as PI3K, mTOR, and the MAPKs via protein–protein interactions [11, 13, 14]. Apart from cell proliferation, IGF2BP1 is also known to regulate tumor cell migration through MK5 and PTEN signaling [15].

MAPKs are central signaling molecules which convey upstream signals from the cell membrane to the nucleus. MAPKs mediate a wide range of cellular processes including cell proliferation, differentiation, and death, of which p38 MAPKs are mainly involved in mediating various signals such as UV radiation, osmotic shock, and inflammatory cytokines. Upon receiving signals from upstream kinases, they interact with specific proteins and activate them by means of phosphorylation. The downstream proteins are usually transcription factors that modulate the gene expression resulting in physiological changes in the cells to respond for the signal. All known MAPK-interacting proteins use ‘D’ domain $(- (R/K)_{1-2} - (X)_{2-6} - \phi - X - \phi -)$ to interact with MAPKs and the interacting proteins are phosphorylated by MAPKs at proline-directed serine/threonine residues [16]. Deregulation of p38 MAPK signaling is known to result in various pathological conditions like cancer, Alzheimer’s, cardiovascular dysfunction, and chronic inflammatory diseases [17]. RNA-binding proteins are known to be regulated through p38 MAPK signaling. Tristetraprolin (TTP), a RNA-binding protein known for binding to several cytokine mRNAs such as TNF- α is regulated by p38 MAPK [18]. In an effort to understand the proteins that are regulated by p38 MAPK, interacting proteins of p38 MAPK were immunoprecipitated and identified by mass spectrometric analysis. To our knowledge this is the first study to report IGF2BP1 as a novel interacting protein of p38 MAPK. Unlike other p38 MAPK substrates, IGF2BP1, being an oncofetal protein, and expressed only in cancer cells would be a potential target to develop novel drugs to treat cancer with fewer side effects.

Materials and methods

Cell culture and transfection

HeLa, human cervical cancer cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum. The cells were incubated at 37 °C in a humidified atmosphere with 5% CO₂. A day prior to transfection, the cells were seeded in 100 mm dishes at a density of $1.5\text{--}2 \times 10^6$ cells. The cells

were transfected with 5 μ g of plasmid encoding Flag-tagged p38 MAPK using Lipofectamine 2000 (Life technologies) as per the manufacturer’s protocol and the cells transfected with empty vector served as control.

Co-immunoprecipitation and western blot

After 24 h of transfection, the cells were lysed with Triton-X Lysis buffer (50 mM Tris pH-7.4, 137 mM NaCl, 20% Glycerol, 1% Triton-X, and complete protease inhibitor cocktail with 1 mM PMSF). An aliquot of the lysates was tested for its expression of Flag-p38MAPK by western blot using the antibodies, anti-Flag and anti-p38 (Cell Signaling). Equal quantities of lysates were incubated with FLAG M2 beads (Sigma) with gentle rocking at 4 °C for 4 h to immunoprecipitate FLAG-p38 along with its interacting proteins. FLAG M2 beads incubated with whole cell lysates of vector-transfected cells served as control. At the end of incubation period, the beads were washed thrice with Triton-X lysis buffer. The washed bead pellet containing the immune complex was resuspended in SDS loading buffer and heated at 95 °C for 5 min. The samples were then resolved on a 10% SDS Polyacrylamide gel and silver stained.

Mass spectrometry

The silver-stained bands were excised into 1 mm² pieces. The proteins in the pieces were destained, reduced (DTT), alkylated (iodoacetamide), and subjected to tryptic digestion overnight at 37 °C. After digestion, the supernatant was subjected to LC–MS/MS analysis. The data obtained were processed using protein Discoverer (version 2.1., Thermo Fisher). All the MS/MS data were converted to mgf files and the files were then submitted to Mascot search algorithm (Matrix science, London, UK) and searched against the UniProt human database.

Preparation of molecules and molecular docking

The X-ray crystal structure of p38 MAPK (MAPK14) and IGF2BP1 were retrieved from the Protein Data Bank (PDB). The PDB IDs of MAPK 14 obtained was 1A9U [19] and that of IGF2BP1 was 3KRM [20]. The retrieved protein structures were checked for the presence of ANI-SOU groups and hetero atoms and were excluded from the study. Further, both the protein structures were energy minimized using SWISS PdbViewer [21]. A molecular docking analysis was carried out using pyDock server [22] that predicts the docking based on interaction restraints. Based on the number of D-domains present in IGF2BP1, interaction restraints were prepared separately for each domain. Except for the interaction residue information, all

the other parameters were set to default parameters to have better comparative analysis. For each interaction restraint, ten best-docked poses were obtained. Based on the RMSD, Electrostatic potential, hydrogen bond interactions, desolvation energy, the docked poses were refined to understand the accurate interaction pattern between the two proteins. The protein complex with least RMSD, electrostatic, and better desolvation energy was taken for further analysis. The protein complex was visualized using PyMOL, and the interacting residues of the two proteins were visualized using LigPlot Plus software [23].

Results

Identification of p38 MAPK-interacting proteins

The whole cell lysates from vector (control) and Flag-p38 MAPK-transfected cells were extracted, and the expression of Flag-p38 MAPK was tested by western blot analysis. With FLAG antibody, a single band of 38 kDa was obtained only in lysates of Flag-p38 MAPK-transfected cells and not in control cells (Fig. 1a upper panel). With the same samples, western blot with p38 antibody revealed a light band corresponding to endogenous p38 MAPK and a strong band corresponding to overexpressed FLAG-p38 MAPK (Fig. 1a lower panel). Western blot analysis was carried out using an aliquot of co-immunoprecipitated samples to confirm (a) immunoprecipitation of Flag-p38MAPK and (b) presence of a well-known p38 MAPK-interacting protein, ATF2, in the immune complex. A single band corresponding to Flag-p38 MAPK (Fig. 1b upper panel) and ATF2 (Fig. 1b lower panel) was obtained only in Flag-p38 MAPK-transfected samples and not in vector-transfected samples. After confirming the presence of ATF2 in the immune complex obtained from FLAG-p38 MAPK samples, large quantities of the samples were

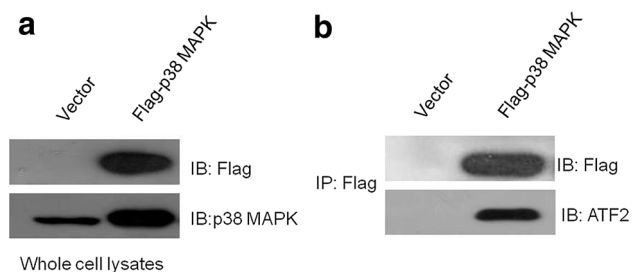


Fig. 1 Analysis of immunoprecipitated samples for presence of ATF2. **a** Western blot analysis was carried out with whole cell lysates obtained from vector and Flag-p38 MAPK-transfected cells. **b** Samples that were immunoprecipitated from these whole cell lysates were analyzed by Western blot. Bands corresponding to Flag-p38 MAPK and ATF-2 were present only in the whole cell lysate from Flag-p38 MAPK-transfected cells and absent in whole cell lysate from vector-transfected samples

separated on a 10% SDS-PAGE along with control immunoprecipitated samples, and the gel was silver stained.

Mass spectrometric analysis

The silver-stained gel was excised into pieces and subjected to trypsin digestion followed by LC–MS/MS analysis. The mass spectrometry results were analyzed on scaffold software. Proteins that are present only in immune complex obtained from Flag-p38 MAPK-transfected cells were considered for further analysis. Also proteins in which minimum of four unique peptides detected in mass spectrometry were considered. IGF2BP1 was considered as a novel p38 MAPK-interacting protein due to (a) the presence of 19 peptides unique to IGF2BP1, (b) the presence of four D-domain, the consensus sequence of MAPK-interacting proteins, and (c) the presence of proline-directed serine/threonine residues (proline directed four threonine residues and one serine residue) (Fig. 2a). The peptides of IGF2BP1 were detected only in the Flag-p38 MAPK-transfected extracts and were absent in the vector-transfected extracts (Fig. 2b).

In silico analysis

The in silico interaction between p38 MAPK (MAPK 14) and IGF2BP1 was analyzed using molecular docking. The docking was performed using pyDock server which utilizes interaction restraints to perform rigid docking. Since there were four D-domains present in the receptor protein (IGF2BP1), four different docking analyses were performed to obtain the best-docked complex. Each docking analysis produced ten docked complexes, and the complex with least RMSD, electrostatic potential, and better desolvation energy was taken for further analysis (Tables 1, 2, 3, 4). Primarily, the docked poses with high binding energy and an RMSD value more than 1.0 Å were excluded from the study. While comparing the energy values obtained from the docking analysis of the four D-domains, we observed least energies to be found with D-Domain 2 (Lys190, Gln191, Gln192, Gln193, Val194, Asp195, Ile196, Pro197, Leu198) (Table 2), thereby interpreting that, there could be least binding affinity of this domain with MAPK14 protein. We selected the top scores of each docked complex and a comparison between the four complexes was made (Table 5). Consequently, we obtained four docked complexes for each docking. When analyzing the best-docked complex, we observed that, IGF2BP1 protein interacted with MAPK14 with Arg433, Phe434, Ala435, Ser436, and Ser437 of the D-domain. Apart from this, there were also other interacting residues of IGF2BP1 and MAPK14 which have been tabulated in Table 6.

sp|Q9NZI8|IF2B1_HUMAN (100%), 63,480.5 Da

Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2

16 exclusive unique peptides, 17 exclusive unique spectra, 19 total spectra, 204/577 amino acids (35% coverage)

a

```

MNK LYIGNLN ESVTPADLEK VFAEHKISYS GQFLVKSGYA FVDCPDEHWA MKAIETFSGK
VELQGKRLEI EHSVPKQRS RKIQIRNIPP QLRWEVLDSL LAQYGTVENC EQVNTSEETA
VVNVTYSNRE QTRQAIMKLN GHQLENHALK VSYIPDEQIA QGPENGRGG FGSRGQPRQG
SPVAAGAPAK QQQVDIPLRL LVPTQYVGA I GKEGATIRN ITKQTQSKID VHRKENAGAA
EK AISVHSTP EGCSSACKMI LEIMHKEAKD TKTADDEVPLK I LAHNNFVGR LIGKEGRNLK
KVEQDTETKI TISSLQDLTL YNPERTITVK GAIENCCR AE QEIMKKVREA YENDVAAMSL
QSHLIPGLNL AAVGLFPASS SAVPPPSSV TGAAPYSSFM QAPEQEMVQV FIPAQAVGAI
IGKKGQHIKQ LSRFASASIK IAPPEPDSK VRMVIITGPP EAQFKAQGR I YGK LKEENFF
GPK EEEVKLET HIRVPASAAG RVIGKGGKTV NELQNLTAAE VVVPRDQTPD ENDQVIVKII
GHFYASQMAQ RKIRDILAQV KQQHQKQGSN QAAARRK

```

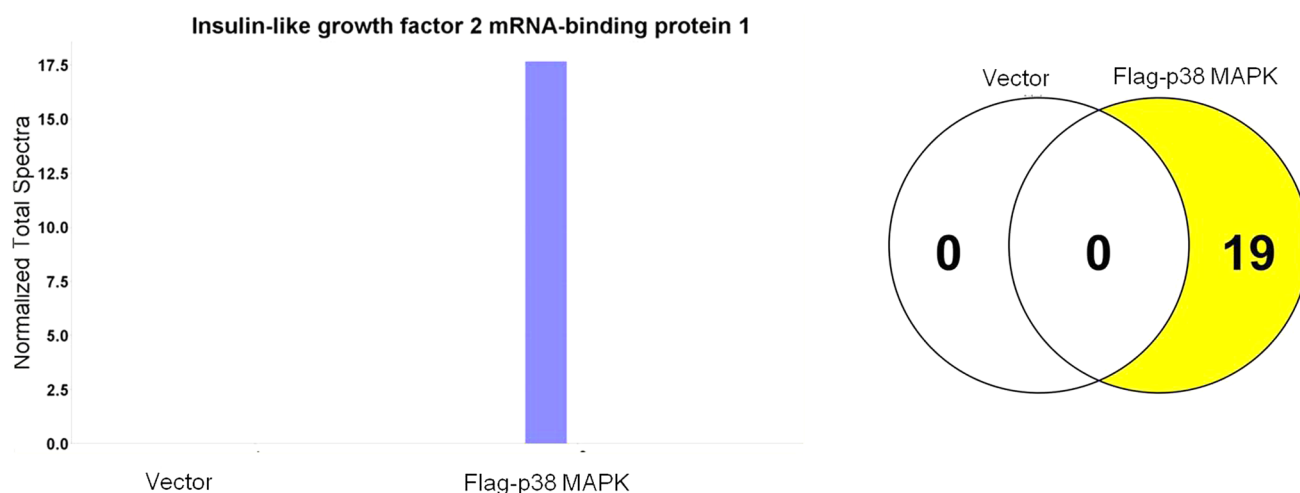


Fig. 2 a Scaffold image of amino acid sequence of IGF2BP1, peptide sequences detected by mass spectrometric analysis are highlighted in yellow. The D-motif sequences are highlighted in gray and the proline-directed serine/threonine residues in black. **b** Scaffold analysis of mass spectra, *Bar graph* Quantity of IGF2BP1 specific peptides spectra were normalized to total spectra. There were no detectable IGF2BP1-related peptides in vector and a value of 17.5

was obtained in F-p38 samples. *Venn diagram* indicating the presence of 19 peptides corresponding to IGF2BP1 in only FLAG-p38 MAPK samples and not in vector samples. *F-p38/FLAG-p38MAPK* Immune complex precipitated from whole cell lysate of Flag-p38 expression vector-transfected cells. *Vector* immune complex from whole cell lysate of empty vector-transfected cells. (Color figure online)

Table 1 Docking predictions of the top 10 models with D-Domain-1 (Lys77, Gln78, Arg79, Ser80, Arg81, Lys82, Ile83, Gln84, Ile85)

Rank of docked pose	RMSD value (Å)	Binding energy(kcal/mol)	Electrostatic force (kcal/mol)	Van der Waals forces (kcal/mol)	Desolvation energy
1	0.87	-22.951	-37.343	42.891	-7.475
2	0.88	-38.854	-43.724	22.334	-1.983
3	0.74	-16.748	-52.06	21.784	9.572
4	0.72	-38.473	-55.21	-4.938	15.461
5	0.82	-50.886	-50.32	37.278	6.362
6	0.347	-32.744	-39.369	8.739	-1.663
7	0.443	-38.193	-46.33	-34.855	9.681
8	0.576	-26.362	-24.602	24.203	-17.904
9	0.882	-40.581	-26.678	36.939	-17.094
10	0.345	-24.116	-48.038	55.078	2.473

Although interacting residues are important, more critical are the various interactions such as van der Waal's forces, electrostatic interactions. This docked conformation was

then subjected to other interaction analysis such as van der Waals and electrostatic potentials as these are a few important interaction [24, 25]. We observed that, Ser436 of

Table 2 Docking predictions of the top 10 models with D-Domain-2 (Lys190, Gln191, Gln192, Gln193, Val194, Asp195, Ile196, Pro197, Leu198)

Rank of docked pose	RMSD value (Å)	Binding energy (kcal/mol)	Electrostatic force (kcal/mol)	Van der Waals forces (kcal/mol)	Desolvation energy
1	0.889	−28.695	−33.031	50.583	−11.897
2	0.873	−36.69	−47.424	−3.656	8.194
3	0.73	−27.336	−54.352	36.228	11.191
4	0.82	−33.726	−32.557	21.643	−9.037
5	1.201	−38.921	−32.227	56.853	−12.832
6	0.76	−36.357	−23.516	20.043	−17.858
7	0.78	−40.243	−54.058	−2.422	14.997
8	0.99	−34.625	−25.241	68.051	−20.804
9	0.87	−39.539	−29.266	50.296	−14.988
10	0.82	−31.276	−46.492	−23.999	9.737

Table 3 Docking predictions of the top 10 models with D-Domain-3 (Arg433, Phe434, Ala435, Ser436, Ala437, Ser438, Ile439, Lys440, Ile441)

Rank of docked pose	RMSD value (Å)	Binding energy	Electrostatic force (kcal/mol)	Van der Waals forces (kcal/mol)	Desolvation energy
1	0.73	−56.445	−67.248	4.621	30.823
2	0.77	−38.676	−66.612	8.686	27.067
3	0.79	−37.848	−64.803	18.4	25.116
4	0.72	−52.156	−62.799	31.935	7.449
5	0.74	−27.434	−62.601	−4.356	35.603
6	0.738	−26.986	−62.219	−3.834	35.617
7	0.829	−35.963	−60.447	61.595	−2.157
8	0.81	−37.248	−60.045	24.151	20.382
9	0.833	−28.935	−59.57	51.16	25.52
10	0.71	−28.734	−59.282	9.588	29.59

Table 4 Docking predictions of the top 10 models with D-Domain-4 (Lys450, Val451, Arg452, Met453, Val454, Ile455, Ile456)

Rank of docked pose	RMSD value (Å)	Binding energy (kcal/mol)	Electrostatic force (kcal/mol)	Van der Waals forces (kcal/mol)	Desolvation energy
1	0.83	−31.342	−30.587	37.752	−11.416
2	1.0	−19.86	−44.15	42.49	1.709
3	0.77	−32.709	−23.996	58.621	−20.056
4	0.73	−22.189	−23.165	−6.723	−14.231
5	0.78	−40.31	−32.895	−29.981	−1.997
6	0.88	−37.351	−64.803	18.4	25.116
7	0.76	−32.033	−41.215	37.526	−0.364
8	0.34	−25.105	−50.414	49.601	7.653
9	0.48	−21.48	−47.771	−2.676	10.261
10	0.69	−38.049	−17.664	45.352	−24.623

IGF2BP1 protein showed strong interactions with Ser326, Asp324, and Arg330 of the p38 MAPK (MAPK14) through one hydrogen bond with each amino acid. In addition, Lys

465 of IGF2BP1 protein formed hydrogen bond with Asp324 and Ala 435 of IGF2BP1 and Arg320 of p38 MAPK (Fig. 3). Electrostatic interactions play a crucial

Table 5 Top scores of docking with the 4 different D-domains

Rank of docked pose	RMSD value (Å)	Binding energy (kcal/mol)	Electrostatic force (kcal/mol)	Van der Waals forces (kcal/mol)	Desolvation energy
1	0.72	−38.473	−55.21	−4.938	15.461
2	0.78	−40.243	−54.058	−2.422	14.997
3	0.73	−56.445	−67.248	4.621	30.823
4	0.34	−25.105	−50.414	49.601	7.653

Table 6 The interacting residues of MAPK14 and IGF2BP1

Proteins	MAPK14	IGF2BP1
Interacting residues	Arg73, His 77, Asp324, Ser326, Phe327, Arg330, Ser329, Asp331, Leu332, Glu336, Ser347, Leu340	Gln405, Lys429, Arg433, Phe434, Ala435, Ser436, Ser438, Pro459, Glu461, Ala462, Lys465

role in many biological processes such as protein–protein interaction, protein–ligand interaction [26–28]. Hence, comparing the number of hydrogen bonds and the electrostatic potential, we observed better binding between the two proteins using D-Domain-3 “RFASASIKI” (Arg433, Phe434, Ala435, Ser436, Ala437, Ser438, Ile439, Lys440, Ile441).

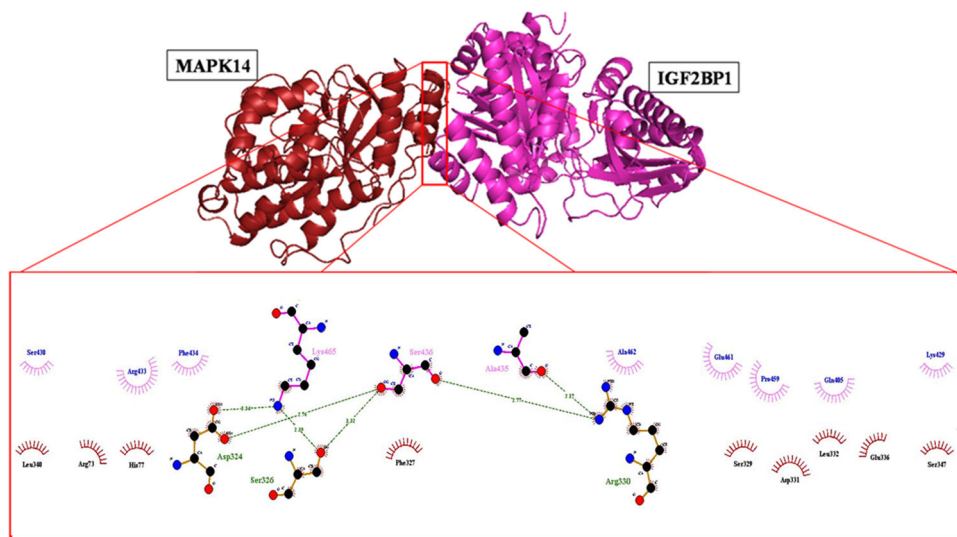
Discussion

Cellular physiology is mainly controlled by interactions between proteins, protein–DNA, and protein–RNA. RNA-binding proteins regulate RNA half-life in the cytoplasm and thus determine the protein levels in the cells there by playing an important role in cellular physiology [29]. In response to cellular stress, p38MAPK interacts with specific proteins in the cytoplasm, leading to the changes in protein composition in the cells, in a way to respond to the

physiological stimuli [30]. p38 MAPK signaling is involved in patho-physiological process such as inflammation, cell division, cancer, metastasis, DNA damage. [31]. Depending upon the cell’s requirement, p38 MAPK mediates its effect by interacting with specific regulatory proteins such as transcription factors, there by changing the protein composition in the cell to respond to the physiological condition. Identification of proteins that interact with p38 MAPK helps us to understand the role played by different interacting partners in various signaling pathways.

The main objective of the present study was to identify the novel proteins that are interacting with p38 MAPK which was not pertained to be cell line specific. In the process we had chosen HeLa cells, which are the most common cell line used for various researches. Its rapid cell proliferating ability in comparison to other cell lines has an advantage for performing the expression studies [32]. Presence of single band in the western blot analysis of whole cell extract using Flag antibody confirms the

Fig. 3 Docked complex MAPK14 (Red) and IGF2BP1 (Pink) protein visualized using PyMOL software. Further the interacting residues and interaction patterns were elucidated with LigPlot Plus software. (Color figure online)



expression of Flag-p38MAPK (Fig. 1a). Immunoprecipitation of Flag-p38MAPK and its interacting proteins (ATF2) was confirmed by western blot analysis (Fig. 1b). Presence of ATF2 in the Flag-p38MAPK-immunoprecipitated samples suggests that other p38 MAPK-interacting proteins are also present in the obtained immune complex. Mass spectrometric-mediated detection of peptides corresponding to a particular protein in the pool of peptides derived from p38MAPK immune complex indicates the presence of that protein in the complex. Identification of peptides corresponding to IGF2BP1 suggests the presence of this protein in the p38 MAPK immune complex. Confidence level increases as the number of peptides detected from a particular protein increases. Presence of 19 different peptides corresponding to IGF2BP1 in the pool of peptides analyzed suggests that this protein is a novel p38 MAPK-interacting protein. The peptide detected corresponds to 35% of the total length of the protein (Fig. 2a). Absence of these peptides in the peptide pool generated from mock immune complex; rules out the possibility of non-specific co-precipitation of IGF2BP1 (Fig. 2b). All proteins that interact with MAPKs use a specific domain known as ‘D’ domain [16]. Presence of four ‘D’ domains in the IGF2BP1 further confirms the possibility of this being a MAPK-interacting protein. All MAPKs are known to regulate downstream signaling molecules by phosphorylating at either one or more proline-directed serine or threonine residues [33]. A scan of IGF2BP1 amino acid sequence for MAPK phosphorylation moiety reveals one serine (Ser 181) and four threonine residues (Thr24, Thr249, Thr446, Thr528,) suggesting these residues may be the site for MAPK phosphorylation (Fig. 2a). Since the Flag-p38 MAPK immune complex was isolated from whole cell lysates obtained using Triton-X lysis buffer which does not disrupt the nuclear membrane, the whole cell lysate consists of proteins from cytoplasmic fraction of the cell and therefore the p38 MAPK-IGF2BP1 interaction is expected to occur in the cytoplasm.

Molecular docking studies are important to understand the various biological interactions between the molecules which may further aid in understanding the biological process. Subsequently, molecular docking protocols offer insights over the fundamental interactions in the protein and between the proteins [34, 35]. In silico analysis of p38 MAPK-IGF2BP1 reveals a feasible complex and four best-docked poses were obtained with least binding energy and RMSD value. Electrostatic potential, van der Waal’s forces, and hydrogen bonds are the other important parameters which determine the interacting residues. Of the four docked poses, the best pose for the interaction between p38 MAPK and IGF2BP1 was observed for D-Domain 3 “RFASASIKI” (Arg433, Phe434, Ala435, Ser436, Ala437, Ser438, Ile439, Lys440, Ile441). The possibility of

IGF2BP1 to affect the kinase activity of p38 MAPK could be eliminated due to the fact that p38 MAPK is upstream in the hierarchy of cell signaling.

Conclusion

Identification of protein–protein interactions responsible for cause of particular disease serves as target for developing novel drugs [36]. IGF2BP1 is overexpressed in several cancers and also associates with metastasis [37]. Developing a cancer-treating drug with minimal side effects is one of the current challenges in the field of medicine. IGF2BP1 has a role in protecting RNA encoding proteins involved in/responsible for causing cancer [12, 38]. Our observation that p38 MAPK interacts with IGF2BP1 suggests that, p38 has a role in IGF2BP1 function. Usually MAPKs activate their substrate by phosphorylation and if experimental evidences can be obtained that IGF2BP1 function is regulated by p38 MAPK, developing drugs to block these interactions would serve as one of the targets for the development of drugs to treat cancer. In this regard, it is pertinent to note that p38 MAPK is also involved in several forms of cancer and metastasis [39]. Inhibitors of p38 MAPK cannot be a choice for treating cancer for the reason that this protein is involved in more than one function; hence blocking p38 MAPK as such would result in side effects. In this regard, it is also important to note that in adults, IGF2BP1 is predominantly expressed only in cancer cells and therefore drugs blocking IGF2BP1–p38 MAPK would act only on cancer cells specifically. Our lab is currently working to establish a role for p38 MAPK in IGF2BP1 function.

Acknowledgements This study was funded by the Department of Biotechnology, Govt. of India (Grant No: BT/PR6207/GBD27/381/2012) (M. A). R.J is a Maulana Azad National Fellow of University Grants Commission, Govt. of India.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

References

1. Venigalla RKC, Turner M (2012) RNA binding proteins as a point of convergence of the PI3K and p38MAPK pathways. *Front Immunol* 3:398. doi:10.3389/fimmu.2012.00398
2. GhignaC Cartegni L, Jordan P, Paronetto MP (2015) Posttranscriptional regulation and RNA binding proteins in cancer biology. *Biomed Res Int* 2015:897821. doi:10.1155/2015/897821
3. Ross AF, Oleynikov Y, Kislauskis EH, Taneja KL, Singer RH (1997) Characterization of a β -actin mRNA zipcode-binding protein. *Mol Cell Biol* 17:2158–2165

4. Havin L, Git A, Elisha Z, Oberman F, Yaniv K, Schwartz SP, Standart N, Yisraeli JK (1998) RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization. *Genes Dev* 12:1593–1598. doi:[10.1101/gad.12.11.1593](https://doi.org/10.1101/gad.12.11.1593)
5. Doyle GA, Betz NA, Leeds PF, Fleisig AJ, Prokipcak RD, Ross J (1998) The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins. *Nucleic Acids Res* 26:5036–5044
6. Leeds P, Kren BT, Boylan JM, Betz NA, Steer CJ, Gruppiso PA, Ross J (1997) Developmental regulation of CRD-BP, an RNA-binding protein that stabilizes c-myc mRNA in vitro. *Oncogene* 14(11):1279–1286. doi:[10.1038/sj.onc.1201093](https://doi.org/10.1038/sj.onc.1201093)
7. Ross J, Lemm I, Berberet B (2001) Overexpression of an mRNA binding protein in human colorectal cancer. *Oncogene* 20:6544–6550. doi:[10.1038/sj.onc.1204838](https://doi.org/10.1038/sj.onc.1204838)
8. Ioannidis P, Trangas T, Dimitriadis E, Samiotaki M, Kyriazoglou I, Tsiapalis CM, Kittas C, Agnantis N, Nielsen FC, Nielsen J, Christiansen J, Pandis N (2001) C-myc and IGF-II mRNA-binding protein (CRD BP/IMP-1) in benign and malignant mesenchymal tumours. *Int J Cancer* 94(4):480–484
9. Ioannidis P, Mahaira L, Papadopoulou A, Teixeira MR, Heim S, Andersen JA, Evangelou E, Dafni U, Pandis N, Trangas T (2003) 8q24 copy number gains and expression of the c-myc mRNA stabilizing protein CRD-BP in primary breast carcinomas. *Int J Cancer* 104(1):54–59. doi:[10.1002/ijc.10794](https://doi.org/10.1002/ijc.10794)
10. Bernstein PL, Herrick DJ, Prokipcak RD, Ross J (1992) Control of c-myc mRNA half-life in vitro by a protein capable of binding to a coding region stability determinant. *Genes Dev* 6:642–654. doi:[10.1101/gad.6.4.642](https://doi.org/10.1101/gad.6.4.642)
11. Dai N, Rapley J, Angel M, Yanik MF, Blower MD, Avruch J (2011) mTOR phosphorylates IMP2 to promote IGF2 mRNA translation by internal ribosomal entry. *Genes Dev* 25(11):1159–1172. doi:[10.1101/gad.2042311](https://doi.org/10.1101/gad.2042311)
12. Bell JL, Wachter K, Muhleck B, Pazaitis N, Kohn M, Lederer M, Huttelmaier S (2013) Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? *Cell Mol Life Sci* 70:2657–2675. doi:[10.1007/s00018-012-1186-z](https://doi.org/10.1007/s00018-012-1186-z)
13. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC (1999) A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol* 19:1262–1270. doi:[10.1128/MCB.19.2.1262](https://doi.org/10.1128/MCB.19.2.1262)
14. Suvasini R, Shruti B, Thota B, Shinde SV, Friedmann-Morvinski D, Nawaz Z, Prasanna KV, Thennarasu K, Hegde AS, Arivazhagan A, Chandramouli BA, Santosh V, Somasundaram K (2011) Insulin growth factor-2 binding protein 3 (IGF2BP3) is a glioblastoma-specific marker that activates phosphatidylinositol 3-kinase/mitogen-activated protein kinase (PI3K/MAPK) pathways by modulating IGF-2. *J Biol Chem* 286(29):25882–25890. doi:[10.1074/jbc.M110.178012](https://doi.org/10.1074/jbc.M110.178012)
15. Stohr N, Kohn M, Lederer M, Glass M, Reinke C, Singer RH, Huttelmaier S (2012) IGF2BP1 promotes cell migration by regulating MK5 and PTEN signaling. *Genes Dev* 26(2):176–189. doi:[10.1101/gad.177642.111](https://doi.org/10.1101/gad.177642.111)
16. Tanoue T, Adachi M, Moriguchi T, Nishida E (2000) A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat Cell Biol* 2:110–116. doi:[10.1038/35000065](https://doi.org/10.1038/35000065)
17. Cuenda A, Rousseau S (2007) p38 MAP-Kinases pathway regulation, function and role in human diseases. *Biochim Biophys Acta* 1773:1358–1375. doi:[10.1016/j.bbamcr.2007.03.010](https://doi.org/10.1016/j.bbamcr.2007.03.010)
18. Brook M, Tchen CR, Santalucia T, McIlrath J, Arthur JS, Saklatvala J, Clark AR (2006) Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. *Mol Cell Biol* 26:2408–2418. doi:[10.1128/MCB.26.6.2408-2418.2006](https://doi.org/10.1128/MCB.26.6.2408-2418.2006)
19. Wang Z, Canagarajah BJ, Boehm JC, Kassisà S, Cobb MH, Young R, Abdel-Meguid S, Adams JL, Goldsmith EJ (1998) Structural basis of inhibitor selectivity in MAP kinases. *Structure* 6:1117–1128. doi:[10.1016/S0969-2126\(98\)00113-0](https://doi.org/10.1016/S0969-2126(98)00113-0)
20. Chao JA, Patskovsky Y, Patel V, Levy M, Almo SC, Singer RH (2010) ZBP1 recognition of beta-actin zipcode induces RNA looping. *Genes Dev* 24(2):148–158. doi:[10.1101/gad.1862910](https://doi.org/10.1101/gad.1862910)
21. Guex N, Peitsch MC (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 18:2714–2723. doi:[10.1002/elps.1150181505](https://doi.org/10.1002/elps.1150181505)
22. Grosdidier S, Pons C, Solernou A, Fernández-Recio J (2007) Prediction and scoring of docking poses with pyDock. *Proteins* 69(4):852–858. doi:[10.1002/prot.21796](https://doi.org/10.1002/prot.21796)
23. Laskowski RA, Swindells MB (2011) LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J Chem Inf Model* 51(10):2778–2786. doi:[10.1021/ci200227u](https://doi.org/10.1021/ci200227u)
24. Smith GR, Sternberg MJE (2002) Prediction of protein–protein interactions by docking methods. *Curr Opin Struct Biol* 12:28–35. doi:[10.1016/S0959-440X\(02\)00285-3](https://doi.org/10.1016/S0959-440X(02)00285-3)
25. Thirumal Kumar D, George Priya Doss C, Sneha P, Tayubi IA, Siva R, Chakraborty C, Magesh R (2016) Influence of V54M mutation in giant muscle protein titin: a computational screening and molecular dynamics approach. *J Biomol Struct Dyn*. doi:[10.1080/07391102.2016.1166456](https://doi.org/10.1080/07391102.2016.1166456)
26. Bashford D (2004) Macroscopic electrostatic models for protonation states in proteins. *Front Biosci* 9:1082–1099
27. Honig B, Nicholls A (1995) Classical electrostatics in biology and chemistry. *Science* 268:1144–1149. doi:[10.1126/science.7761829](https://doi.org/10.1126/science.7761829)
28. Warshel A, Aqvist J (1991) Electrostatic energy and macromolecular function. *Annu Rev Biophys Biophys Chem* 20:267–298
29. Sunnerhagen P (2007) Cytoplasmic post-transcriptional regulation and intracellular signaling. *Mol Genet Genom* 277(4):341–355. doi:[10.1007/s00438-007-0221-5](https://doi.org/10.1007/s00438-007-0221-5)
30. Zarubin T, Han J (2005) Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 15:11–18. doi:[10.1038/sj.cr.7290257](https://doi.org/10.1038/sj.cr.7290257)
31. Cuadrado A, Nebreda AR (2010) Mechanisms and functions of p38 MAPK signalling. *Biochem J* 429(3):403–417. doi:[10.1042/BJ20100323](https://doi.org/10.1042/BJ20100323)
32. Lucey BP, Nelson-Rees WA, Hutchins GM (2009) Henrietta Lacks, HeLa cells, and cell culture contamination. *Arch Pathol Lab Med* 133(9):1463–1467. doi:[10.1043/1543-2165-133.9.1463](https://doi.org/10.1043/1543-2165-133.9.1463)
33. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 22(2):153–183. doi:[10.1210/edrv.22.2.0428](https://doi.org/10.1210/edrv.22.2.0428)
34. Vakser IA (2014) Protein–protein docking: from interaction to interactome. *Biophys J* 107:1785–1793. doi:[10.1016/j.bpj.2014.08.033](https://doi.org/10.1016/j.bpj.2014.08.033)
35. Sneha P, Doss CG (2016) Gliptins in managing diabetes—Reviewing computational strategy. *Life Sci* 166:108–120. doi:[10.1016/j.lfs.2016.10.009](https://doi.org/10.1016/j.lfs.2016.10.009)
36. Jacob R, Anbalagan M (2016) Targeting secret handshakes of biological processes for novel drug development. *Front Biol* 11(2):132–140. doi:[10.1007/s11515-016-1394-2](https://doi.org/10.1007/s11515-016-1394-2)
37. Yaniv K, Yisraeli JK (2002) The involvement of a conserved family of RNA binding proteins in embryonic development and carcinogenesis. *Gene* 287:49–54. doi:[10.1016/S0378-1119\(01\)00866-6](https://doi.org/10.1016/S0378-1119(01)00866-6)
38. Stohr N, Lederer M, Reinke C, Meyer S, Hatzfeld M, Singer RH, Huttelmaier S (2006) ZBP1 regulates mRNA stability during cellular stress. *J Cell Biol* 175(4):527–534. doi:[10.1083/jcb.200608071](https://doi.org/10.1083/jcb.200608071)
39. Igea A, Nebreda AR (2015) The Stress Kinase p38 α as a Target for Cancer Therapy. *Cancer Res* 75(19):3997–4002. doi:[10.1158/0008-5472.CAN-15-0173](https://doi.org/10.1158/0008-5472.CAN-15-0173)