Designing Therapeutic Peptides for Inhibiting the mTORC1 Signalling Pathway: A Novel Trend in Cancer Therapeutics

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Abstract: Cancer is the second leading cause of death globally, characterized by hallmark features including uncontrolled cell proliferation, enhanced survival, abnormal angiogenesis, immune evasion, and metabolic reprogramming. The mTOR signalling pathway intersects with all these hallmarks, positioning it as an attractive therapeutic target. One promising approach involves disrupting protein-protein interactions using peptides derived from interfacial amino acids of binding partners. This study employed such a strategy to inhibit the association between mTOR kinase and RHEB, a critical interaction for mTORC1 activation. Three native peptides were designed from the mTOR-RHEB interface sequence. These were further modified by incorporating evolutionarily conserved mutations observed in mTOR sequences. All peptides were evaluated for binding affinity with RHEB through protein-peptide docking using HADDOCK 2.4 and binding energy calculations via FoldX5.0. Interaction analyses were performed using LigPlot+. The peptides were designated N1-3 (native), M1-3 (mutated), and C1-3 (control). Among these, peptides M2 and M3 demonstrated binding energies of −14.28 kcal/mol and −14.53 kcal/mol, respectively, comparable to the complete mTOR-RHEB complex (−15.83 kcal/mol). These findings were supported by extensive hydrogen bonding and favourable intermolecular interactions. The results suggest that these peptides possess significant potential to disrupt mTORC1 signalling, warranting further validation through in vitro studies for cancer therapeutic applications.

1. Introduction

Cancer remains one of the leading causes of death worldwide, with prostate, breast, and lung cancer being the most commonly diagnosed types. Alarmingly, both the incidence and mortality rates of cancer continue to rise at an unprecedented pace [1,2]. A key driver of cancer progression is the hyperactivation of critical cellular signalling pathways, including the Ras/Raf, PI3K/AKT, and mammalian Target of Rapamycin (mTOR) pathways. Among these, the mTOR pathway ranks as the second most frequently dysregulated pathway in human cancers, following only the p53 pathway [3,4].

At the heart of this pathway lies mTOR, a serine/threonine-protein kinase that serves as a central regulator of cellular functions. mTOR functions as part of two distinct multi-protein complexes: mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2). mTORC1 is composed of mTOR kinase, RAPTOR (a scaffolding protein), PRAS40 (a negative regulator), mLST8/GβL (which enhances kinase activity), and DEPTOR (another negative regulator). In contrast, mTORC2 includes mTOR kinase, RICTOR (a scaffolding protein), PROTOR1/2, mSIN1, mLST8/GβL, and DEPTOR (Fig. 1). These complexes govern a wide array of essential biological processes, including cell survival, growth, metabolism, protein synthesis, proliferation, autophagy, and overall cellular homeostasis. Notably, each complex performs these functions through distinct mechanisms [5].

Various environmental and cellular signals finely tune the activity of the mTOR pathway. These include nutrient availability (such as amino acid levels), oxygen and glucose concentrations, cellular energy status (ATP levels), hormones, growth factors, and various forms of cellular stress. These signals modulate the pathway by either activating or suppressing key transcription factors and proteins through their respective receptors, ultimately determining cellular responses to changing conditions (Fig. 1) [6].

Primarily, three signalling pathways are involved in activating the mTOR pathway (Fig. 1). First, the TSC1/2 complex, which releases the RHEB, gets inhibited by GF-regulated PI3K/AKT pathway allowing it to bind to the GTP. This “GTP-charged” RHEB then activates the mTOR pathway. Further, as the system is in a low energy state, it stimulates the Ser/Thr protein kinase STK11, which is responsible for the phosphorylation of AMPK. This

phosphorylated AMPK causes TSC2-mediated inhibition of mTOR. A third route to regulate mTOR is amino acid-dependent.

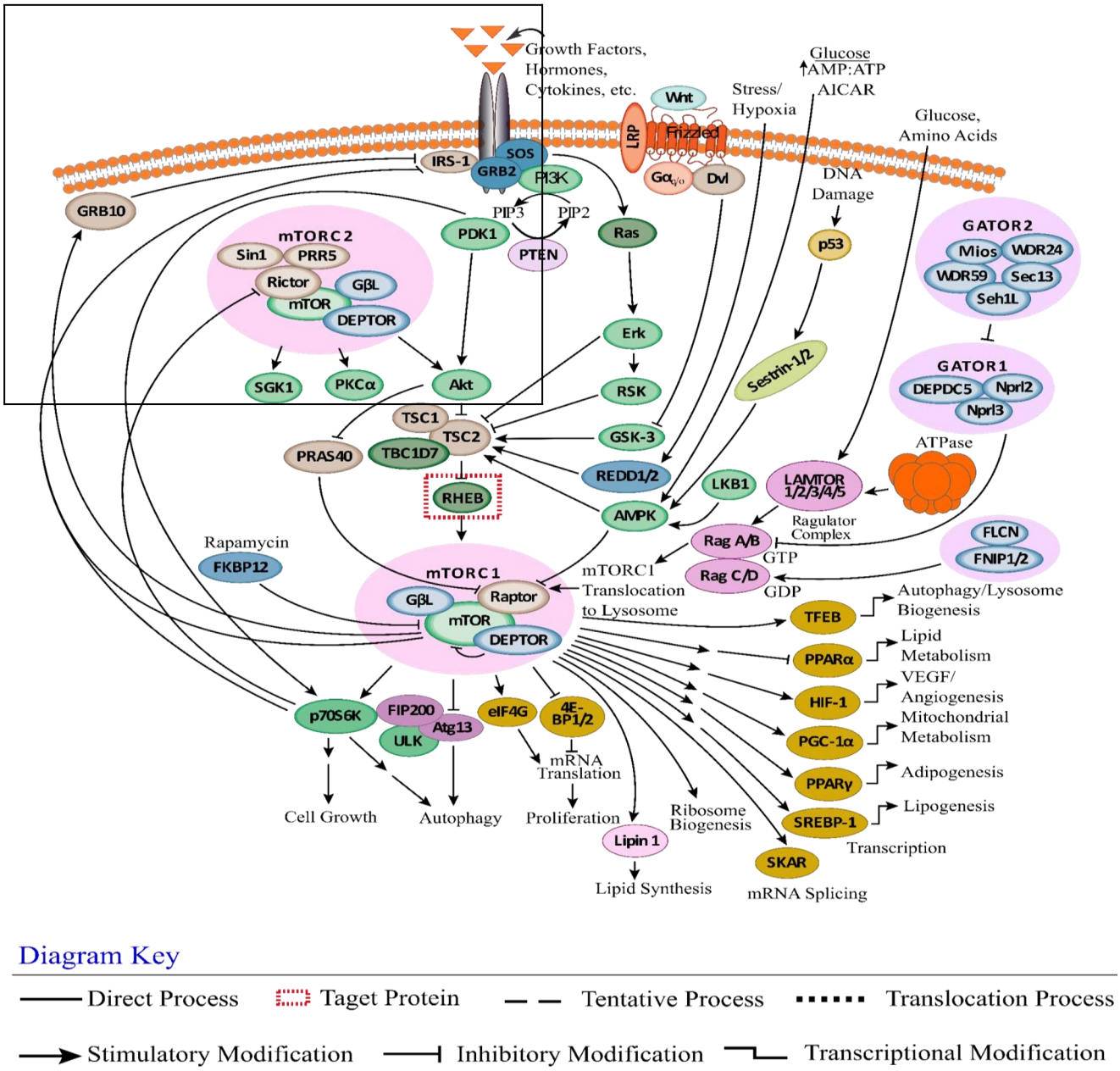

Fig 1: The mTOR signaling pathway integrates nutrient signals and growth factor via RTKs, PI3K/AKT, and TSC1/2 to regulate RHEB. GTP bound RHEB activates mTORC1, promoting protein synthesis through S6K1 and 4E-BP1. Designed peptides targeting RHEB aim to disrupt mTORC1 activation for cancer therapy [5,14, KEGG].

Here, the GATOR1 complex (DEP domain-containing protein 5, DEPDC5; NPRL2 and NPRL3) controls mTOR activity, which is affected by the level of amino acids, importantly via leucine and arginine. When the level of amino acids is low, GATOR1 will cause inhibition of mTOR signalling; when they are in normal range, GATOR1 does not inhibit [7]. Hyperactivation of the mTOR pathway due to gain-of-function mutations and loss-of-function mutations of genes for tumour suppressors like p57, PTPN11, tensin and phosphatase homolog (PTEN), tuberous sclerosis 1/2 (TSC1/2) and neurofibromin 1/2 (NF1/2), is associated with cancer development and progression.

Certain mutations, such as R2505P and S2215Y, in the mTOR kinase are also characterised as carcinogenic [8–10].

Rapamycin is the very first discovered inhibitor of the mTOR pathway; it binds to FK506 Binding Protein 12 (FKBP12) leading to the dissociation of RAPTOR from mTORC1 but also negatively regulates many other proteins such as bFGF, PDGF, VEGF, and so on. A number of allosteric inhibitors of mTOR known as rapalogs (e.g., temsirolimus, everolimus, ridaforolimus, and ABI-009) have also been evaluated as monotherapies as well as combinatorial therapies to target various cancer types, but they are still under preclinical and clinical trials. Also, it is well known that small molecule inhibitors are inefficient to cover the large surface areas of Protein-Protein Interactions (PPIs). Hence, novel inhibitors are needed for mTORC1 inhibition [11].

The other way of inhibiting a protein could be that derivatives of either of the proteins interact with their counterparts and prevent the association between the two proteins, hence inhibiting the action of the proteins. Based on this fact, peptides can be designed as inhibitors, and so, peptide inhibitors have now become the novel choice for PPI inhibition. Peptides with 20–60 residues are the better choice, as small peptides below this size cannot maintain their secondary structures [12,13].

Using the same rationale, in the present study, potential peptides against RHEB were designed using the amino acid sequences of the mTOR kinase from the mTOR-RHEB interfacial region. It is hypothesised that these designed peptides will irreversibly bind to the RHEB and will prevent its binding to the mTOR kinase, thus discouraging the activation of the mTORC1 complex.

2. Materials and Methods

2.1. Prediction of binding affinity at mTOR-RHEB interface

RHEB-bound mTORC1 complex structure (PDB Id: 6BCU) (Cryo-EM structure) was retrieved from the Protein Data Bank (PDB). It has ten chains annotated as mTOR Kinase (Chain A & B), RAPTOR (Chain W & Y), eIF-4EBP1 (Chain X & Z), RHEB (Chain R & S), mLST8 (Chain D & E). Chain A and S were used to predict the mTOR kinase-RHEB interface and the interacting amino acids between them with the help of the Ligplot+ suite for protein-protein interface (PPI) prediction [15]. FoldX5.0 was used to predict free energy change (ΔG) of the mTOR-RHEB complex. The FoldX uses various linear combinations of empirical terms for free energy change (ΔG) calculation, as depicted in the equation given below.

$$\Delta G = a\cdot\Delta Gvdw + b\cdot\Delta GsolvH + c\cdot\Delta GsolvP + d\cdot\Delta Gwb + e\cdot\Delta Ghbond + f\cdot\Delta Gel + g\cdot\Delta Gkno + h\cdot T\Delta Smc + k\cdot T\Delta Ssc + l\cdot T\Delta Sclash \quad \text{— Eq. 1}$$

In this equation, the initial alphabetical characters before each energy term signify its weight in free energy change (ΔG) calculation.

2.2. Identification of Evolutionary Changes and Mutations Retrieval in mTOR kinase

UniProt database and further literature survey was utilized to list experimental and natural mutations in the mTOR and the RHEB. While multiple sequence alignment (MSA) was used to identify the evolutionary changes in the mTOR kinase sequence in different vertebrates. The MSA of 50 randomly selected FASTA sequences of the mTOR kinases, from different vertebrates, was performed on the MEGA-X (Molecular Evolutionary Genetics Analysis X) platform using its Clustal-W algorithm [16]. The evolutionary changes, as observed in the residues from the interacting regions of the N-Heat domain of the mTOR kinase in different vertebrates, are summarised in Table-S1.

2.3. Peptide Designing

The mTOR-RHEB interface prediction revealed that all hydrogen bonds and numerous hydrophobic interactions between mTOR kinase and RHEB were mediated by a specific segment of mTOR's N-Heat domain, spanning amino acid residues 90I to 160R. Based on this finding, we hypothesized that peptides derived from this region could bind irreversibly to RHEB, thereby disrupting interface formation between mTOR kinase and RHEB GTPase.

For peptide design, chain A (mTOR kinase) of the 6BCU structure was used to extract three distinct sequences using UCSF Chimera 1.15r [17]: residues 90I–119D (Native-1, N1), 130I–160R (Native-2, N2), and 101A–154E (Native-3, N3) (Fig. 2). Since these sequences remained unaltered, they were designated as native peptides. Evolutionary changes identified during sequence analysis of mTOR kinase were subsequently introduced into N1,

N2, and N3 as mutations, generating Modified-1 (M1), Modified-2 (M2), and Modified-3 (M3) peptides respectively (Table 1). Additionally, only those evolutionary changes that functioned as constructive or neutral mutations—determined by their individual impact on the mTOR-RHEB complex's ΔG (dΔG) as discussed in results—were incorporated to design Constructive-1 (C1), Constructive-2 (C2), and Constructive-3 (C3) peptides. All nine peptides underwent energy minimization and charge optimization using UCSF Chimera 1.15r [17], with mutations introduced through the same tool. The classification of each evolutionary change as constructive, neutral, or destructive was based on its calculated dΔG value when individually incorporated into the mTOR-RHEB complex.

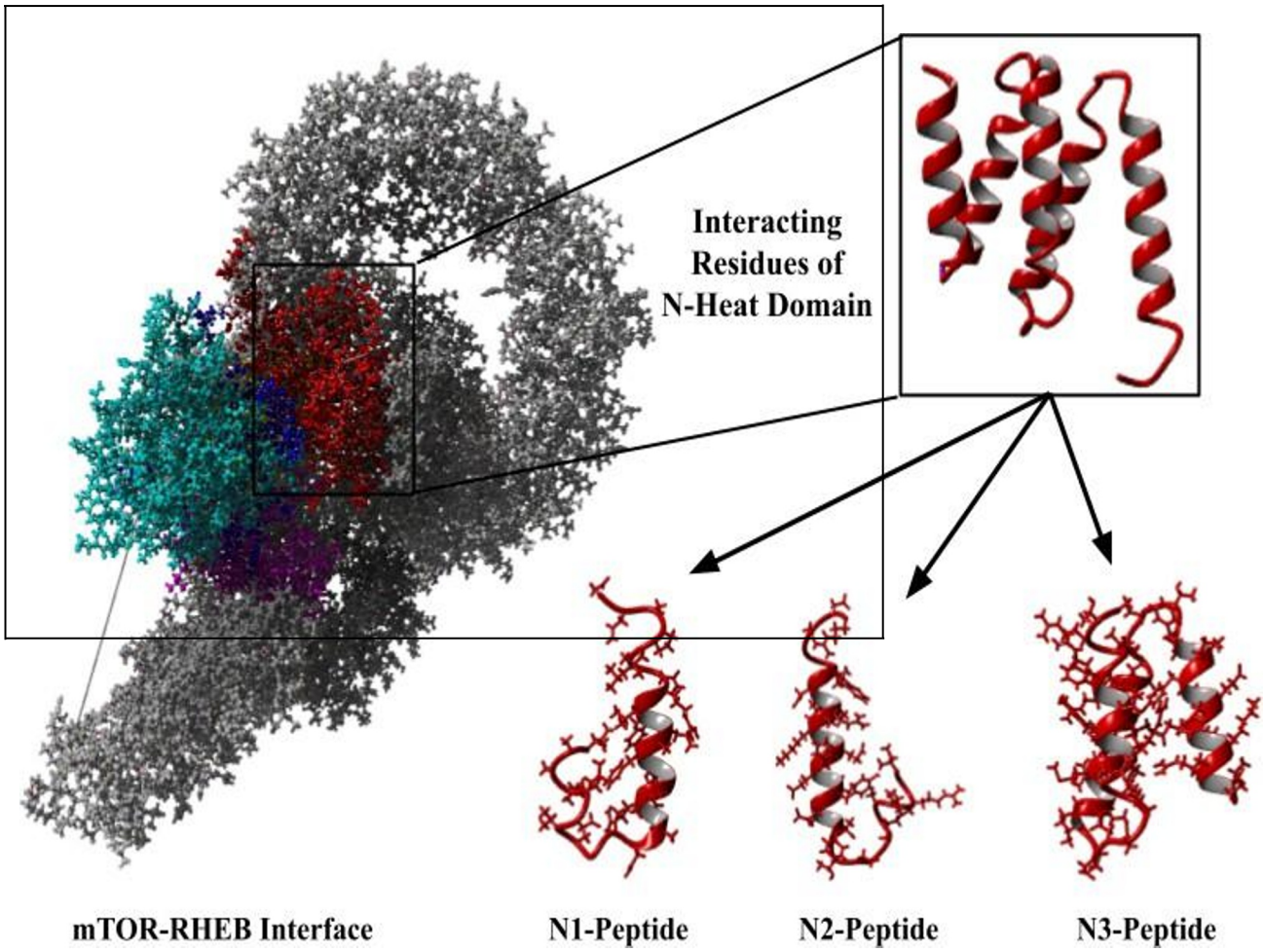

Fig-2: The peptides N1, N2 and N3 were designed by using the amino acid sequences from the N-Heat domain of mTOR kinase. The red colour in the mTOR-RHEB interface depicts the interacting residues of N-Heat, while the blue colour depicts the residues of RHEB which are involved in interface formation. The helices of N-Heat spanning the residues 90I to 119D, 130I to 160R and 101A to 154E were cut apart from the N-Heat of the mTOR kinase and optimized to be used as peptide N1, N2 and N3, respectively. These peptides were further modified to design rest of the peptides i.e. M1, M2, M3, C1, C2 and C3.

Table-1: All the peptides with their amino acid sequences and description of mutations incorporated in them (if any)

Peptide Name	Peptide Sequence	Mutations Incorporated	Peptide length

N1	IASLIGVEGGNATRIGRFANYLRNLLPSND (90I-119D)	—	30
N2	IGRLAMAGDTFTAEYVEFEVKRALEWLGADR (130I-160R)	—	31
N3	ATRIGRFANYLRNLLPSNDPVVMEMASKAIG RLAMAGDTFTAEYVEFEVKRALE (101A-154E)	—	54
M1	IVSLIGVEAGYSTRIGRFANYLRNLLPSSD (90I-119D)	V91A, A98G, Y100N, S101A, S118N	30
M2	MGHLSLTGDTFTAEYVEFEVKRALEWLGADR (130M-160R)	M130I, H132R, S134A, L135M, T136A	31
M3	VTRISRFANYLRNLLPSSDPVVMEMASKAMGHLSLTGDTCTAEYVEFEVKRA LE (101V-154E)	V91A, S105G, S118N, M130I, H132R, S134A, L135M, T136A, C140F	54
C1	IVSLIGVEAGNVTRISRFANYLRNLLPSSD (90I-119D)	V91A, A98G, V101A, S118N	30
C2	MGHLALAGDTFTAEYVEFEVKRALEWLGADR (130M-160R)	M130I, H132R, L135M	31
C3	VTRISRFANYLRNLLPSSDPVVMEMASKAMGHLALAGDTFTAEYVEFEVKRA LE (101V-154E)	V101A, S105G, S118N, M130I, H132R, L135M	54

2.4. Protein-Peptide Docking

The protein-peptide docking was performed on HADDOCK 2.4 (High Ambiguity Driven Biomolecular DOCKing) web portal [18] and the results of the docking interface were further water refined on the refinement interface of HADDOCK 2.4. The free energy changes (ΔG) of the refined RHEB-peptide complexes were analyzed with the FoldX5.0 interface after minimizing the refined complexes [19–25]. The HADDOCK server produced docking results in ten clusters constituting almost 100 models for each RHEB-peptide complex. Out of all these models, only the top four models of each top two clusters were selected for further water refinement and energy calculation. So, eight models for each peptide-RHEB complex were water refined, resulting in four new refined models for each model. Only the topmost model of these four refined models was selected for energy minimization and ΔG calculation. The ΔGs of all eight refined models of each RHEB-peptide complex were calculated and compared among themselves.

3. Results

3.1. Analysis of the mTOR-RHEB Interface

It is observed that mTOR kinase and RHEB interact on the mTOR-RHEB interface. mTOR kinase and RHEB interact predominantly with the help of the N-Heat domain of the mTOR kinase and the switch II (residue 63–79)

of the RHEB. Eight hydrogen bonds along with other interactions are predominantly involved from RHEB with mTOR.

Residues 69H, 101A, 106R, 109N, 112R and 154E (all from the N-Heat domain of the mTOR kinase) are involved in hydrogen bonding with residues 109K, 72G, 105D, 75S, 76I and 7R of the RHEB GTPase, respectively. The remaining residues form other kinds of interactions including salt bridges, hydrophobic interactions, pi-pi bonds, etc. (Fig. S1). The mTOR-RHEB interface analysis showed that in total eight hydrogen bonds are formed between the mTOR kinase and the RHEB, which are solely formed by the N-Heat domain of the mTOR kinase with the RHEB. Also, most of the interfacial contacts are shown by the N-Heat domain of the mTOR kinase with the RHEB (Fig. S1). Hence, the portions of the N-Heat domain were selected as native peptides namely N1, N2 and N3, which were further modified to design the rest of the peptides as summarized in Table-1.

3.2. The evolutionary changes (mutations) and their incorporation into constructing peptides

As mentioned in materials and methods, evolutionary changes in mTOR sequences from different animals were analyzed in the process of designing the best peptide inhibitors. In total, 50 sequences of the mTOR kinase from vertebrates were aligned with the Human mTOR kinase. These evolutionary changes observed in the process were being recorded and briefly summarized in Table-S1.

While analyzing the change in ΔG (dΔG) of interaction between the mTOR and the RHEB, after incorporating each mutation separately, it was noticed that dΔG for some mutations was positive (rows 3, 4, 10, 12 and 13 of Table-S1), zero or there was no change in ΔG for some (rows 1, 2, 7, 8 and 11 of Table-S1) and negative for others (rows 5 and 6 of Table-S1). Here, mutations with positive dΔG indicate destructive mutations while negative dΔG indicates constructiveness of the mutations, and mutations with no change in ΔG are neutral in nature. The constructive mutations improve the association between two interactive partners and help in decreasing ΔG. These calculations were used to construct different peptides as described and tabulated in materials and methods.

3.3. RHEB-peptide Docking

At first, the mTOR kinase and RHEB were docked as a control, followed by newly prepared peptides with the RHEB, using the HADDOCK interface to compare the results. Out of the various docking models formed, the best models for each RHEB-peptide complex were refined and analysed for their ΔG, number of H-bonds, along with other interactions. The best model for each RHEB-peptide complex is noted in Table-2. For example, amongst all models of the RHEB-N1 complex, model CL1.2 (model 2 of cluster 1) was the best, as noted in Table-2.

Table-2: The comparison of RHEB-Peptide complexes in terms of ΔG, hydrogen bonds and other interactions

Complex/Peptide	Lowest ΔG (kcal/mol)	H-Bonds	Total Interactions
mTOR-RHEB/Control	-15.83	8	37
N1	-9.34	8	18
N2	-9.50	4	21
N3	-12.17	8	22
M1	-9.18	8	23
M2	-14.28	7	24
M3	-14.53	8	20
C1	-10.21	7	22
C2	-9.18	5	21
C3	-13.25	11	23

It was observed that peptides M2 and M3 show a better ΔG along with a good number of bonding interactions between the peptides and the RHEB in comparison to other RHEB-Peptide complexes. The ΔG for RHEB-M2 is −14.28 kcal/mol while ΔG for RHEB-M3 is −14.53 kcal/mol, both of which are comparable to the ΔG of the mTOR-RHEB complex which is −15.83 kcal/mol. The RHEB-M2 has seven H-bonds along with 17 other contacts like pi-pi bonds, ionic bonds and salt bridges. Similarly, RHEB-M3 has eight H-bonds along with 12 other contacts. The binding positions of these peptides in the grooves of the RHEB show that they fit very tightly into the grooves (see Fig. 3). These findings highlight the strong potential of peptides M2 and M3 for further investigation and experimental validation.

Another peptide, C3, also shows promising characteristics. Although its binding energy (ΔG = −13.25 kcal/mol) is slightly less favorable compared to M2 and M3, it still demonstrates meaningful interaction with RHEB. The formation of eleven hydrogen bonds along with twelve additional covalent and electrostatic interactions further supports its candidacy as a potential anticancer peptide. Additionally, all three peptides exhibit interface root mean square deviations (i-RMSD) below 2.0 Å, indicating stable and reproducible binding modes. Their HADDOCK scores range from −90 to −70, reflecting favorable docking outcomes (see Supplementary document). Together, these results reinforce the therapeutic potential of these peptides and justify their progression toward experimental validation.

Discussion

Hyperactivation of mTORC1 has often been found to be associated with most of the cancer hallmarks. It has been considered a major therapeutic target to treat these hallmarks and has often been inhibited using small molecular inhibitors. But most of these small inhibitors including rapamycin and rapalogs, have serious side effects and also activate mTORC2 alongside which causes improper functioning of healthy cells too. Hence, the mTORC1-specific target for cancer therapeutics may be a better option. The RHEB-GTPase may be one such option to explore upon. Mahoney in 2018, successfully interrupted the mTORC1 signalling by inhibiting the RHEB GTPase using a small molecular inhibitor named NR1, which successfully bound to the interfacial residues of Switch II of RHEB and prevented its association with the mTOR kinase [26]. The present study suggests that RHEB can be a possible target for inhibiting mTOR cascade efficiently using anticancer peptides.

Anticancer peptide therapy has gained momentum in the last two decades as they are potent enough, specific and safe for the treatment and have the ability to target their protein as they are designed strategically from the interacting domains [27]. Their synthesis is easy and they can be modified according to the need by chemical or biological methods. Protein targets: Ras/NF-κB/c-Myc activation, MAP kinases and p53 are being targeted by various types of inhibitors including peptides, for treating cancerous conditions [28]. One such example is the work of Chung and his colleagues from 1991; they inhibited the Ras-induced maturation of the *Xenopus laevis* oocyte using small peptides from its interface [29,30]. Another study by Barnard et al. 1998, also identified that peptides from similar regions of Ras, including amino acids 32–37 and 40–45, could block the Ras–Raf interaction [31]. The present study aimed at designing peptide inhibitors targeting the RHEB GTPase and analyzing its effectiveness to inhibit the mTORC1 pathway. A similar study has been performed by Shams et al. in 2022, who successfully inhibited the mTORC1 pathway by targeting RHEB GTPase using small peptides from its interfacial region [32].

For the present study it was critical to identify the mTOR-RHEB interface. This surface was previously studied and determined by Yang et al. in 2017. Yang and colleagues mentioned that Switch I (33D-41N), Switch II (63G-79N), residues 5K–7R and 106M–111Q of the RHEB GTPase form interface with the portions of N-Heat domain (60S-157G), M-Heat domain (966H-1020V) and FAT domain (1277K-1307A) of the mTOR kinase [33]. This information was reconfirmed by the results of the mTOR-RHEB interface from LigPlot+.

Followed by this, the peptides were designed to dock with the RHEB GTPase to deduce their potency to inhibit mTOR-RHEB association. For the same, the mutations and the evolutionary changes in the mTOR kinase within vertebrates were recorded to incorporate them as mutations in the designed peptides to enhance them. Surprisingly, most of the evolutionary changes/mutations in the mTOR kinase lay outside the mTOR-RHEB interface, but nearly 40 of the evolutionary changes in the residues were noted at the interface as well (Fig. S2). Here, the term “evolutionary changes” signifies the replacement of one amino acid residue with another amino acid residue at a specific position occurring during the course of evolution. This concept hypothesizes that these evolutionary mutations definitely might have put some changes in the functionality of the protein in the sequence of which they

have occurred, as only the adaptive mutations in the course of evolution remain with the organisms as per the Darwinian theory of survival of the fittest [34].

Both natural and experimentally observed mutations in the mTOR kinase are located away from the mTOR-RHEB interface (Fig. S2A), rendering them unsuitable for peptide design. However, evolutionary mutations occurring in mTOR kinase across different vertebrate species were found within this critical interfacial region (Fig. S2B). These evolutionarily conserved changes informed our peptide enhancement strategy, where corresponding residue modifications were incorporated into native peptide sequences. As anticipated, several newly designed peptides demonstrated improved binding affinity with RHEB.

Despite these promising results, the therapeutic application of such peptides in cancer treatment faces significant hurdles due to inherent pharmacological limitations. Peptides exhibit poor pharmacokinetic properties during in vivo applications, primarily because circulating proteases rapidly degrade them in body fluids. Additionally, their inherent charges and relatively large molecular size impede efficient cellular membrane penetration. Ongoing research efforts are addressing these challenges through advanced peptide delivery systems, structural optimization to enhance secondary conformations, sequence modifications, and various derivatization strategies [35].

4. Conclusions

While designing the anticancer peptides for mTOR in the present study, it has been observed that the mTOR-RHEB interface has 37 bonds, including 8 H-bonds with an overall ΔG of −15.83 kcal/mol. It is concluded from the present study that most of the interactions between the mTOR and RHEB complex are confined to the N-Heat domain of the mTOR kinase and the Switch I & II of the RHEB GTPase. This helped to design the peptides for mTOR-RHEB inhibition. Another approach which was included in this study to design inhibitor peptides was to consider the mutations occurring in the mTOR kinase during evolutionary changes. Out of 3 native and 6 newly designed peptides, M2 and M3 peptides were found to have comparable binding energies to the mTOR-RHEB complex, with the energies −14.28 kcal/mol and −14.53 kcal/mol, respectively, and also have various favorable interactions. One more peptide, C3, with ΔG of −13.25 kcal/mol and 11 H-bonds, can also be considered for further studies. Hence, these peptides can prove their best candidacy for peptide therapy of several cancer types, which is subject to further investigation and experimentation. These peptides can be further explored by in vitro and in vivo tests as potential lead peptides against cancers.

Authors' Contributions

All the authors participated in study design, framing, experimentation, result interpretation and editing the final thesis. AT worked on designing the study, experimentations, result interpretations and manuscript writing. AK designed the experiments, analysed the results and edited the manuscript while RR designed the study, formatted the research, worked on the interpretation part of the study and reviewed it.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Table-S1: The mutations of the mTOR Kinase in the interfacial region of mTOR and RHEB

S.No.	Residue Changes	Associated vertebrate	dΔG kcal/mol
1	91A → V	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	0.0
2	98G → A	*Phascolarctos cinereus, Vombatus ursinus*	0.0
3	100N → Y	*Phascolarctos cinereus, Vombatus ursinus*	0.21
4	101A → S	*Mesocricetus auratus*	0.25
5	101A → V	*Podarcis muralis*	-0.35
6	105G → S	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	-0.36
7	118N → S	*Mesocricetus auratus*	0.0
8	130I → M	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	0.0
9	132R → H	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	0.0
10	134A → S	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	0.05
11	135M → L	*Gopherus agassizii*	0.0
12	136A → T	*Alligator sinensis*	0.02
13	140F → C	*Vombatus ursinus*	0.30

Table-S2: All the peptides with their amino acid sequences and description of mutations incorporated in them (If any)

Peptide Name	Peptide Sequence	Mutations Incorporated	Peptide length
N1	IASLIGVEGGNATRIGRFANYLRNLLPSND (90I-119D)	—	30
N2	IGRLAMAGDTFTAEYVEFEVKRALEWLGADR (130I-160R)	—	31
N3	ATRIGRFANYLRNLLPSNDPVVMEMASKAIGR LAMAGDTFTAEYVEFEVKRALE (101A-154E)	—	54
M1	IVSLIGVEAGYSTRIGRFANYLRNLLPSSD (90I-119D)	V91A, A98G, Y100N, S101A, S118N	30
M2	MGHLSLTGDTFTAEYVEFEVKRALEWLGADR (130M-160R)	M130I, H132R, S134A, L135M, T136A	31
M3	VTRISRFANYLRNLLPSSDPVVMEMASKAMG HLSLTGDTCTAEYVEFEVKRALE (101V-154E)	V91A, S105G, S118N, M130I, H132R, S134A, L135M, T136A, C140F	54
C1	IVSLIGVEAGNVTRISRFANYLRNLLPSSD (90I-119D)	V91A, A98G, V101A, S118N	30
C2	MGHLALAGDTFTAEYVEFEVKRALEWLGAD R (130M-160R)	M130I, H132R, L135M	31
C3	VTRISRFANYLRNLLPSSDPVVMEMASKAMG HLALAGDTFTAEYVEFEVKRALE (101V-154E)	V101A, S105G, S118N, M130I, H132R, L135M	54

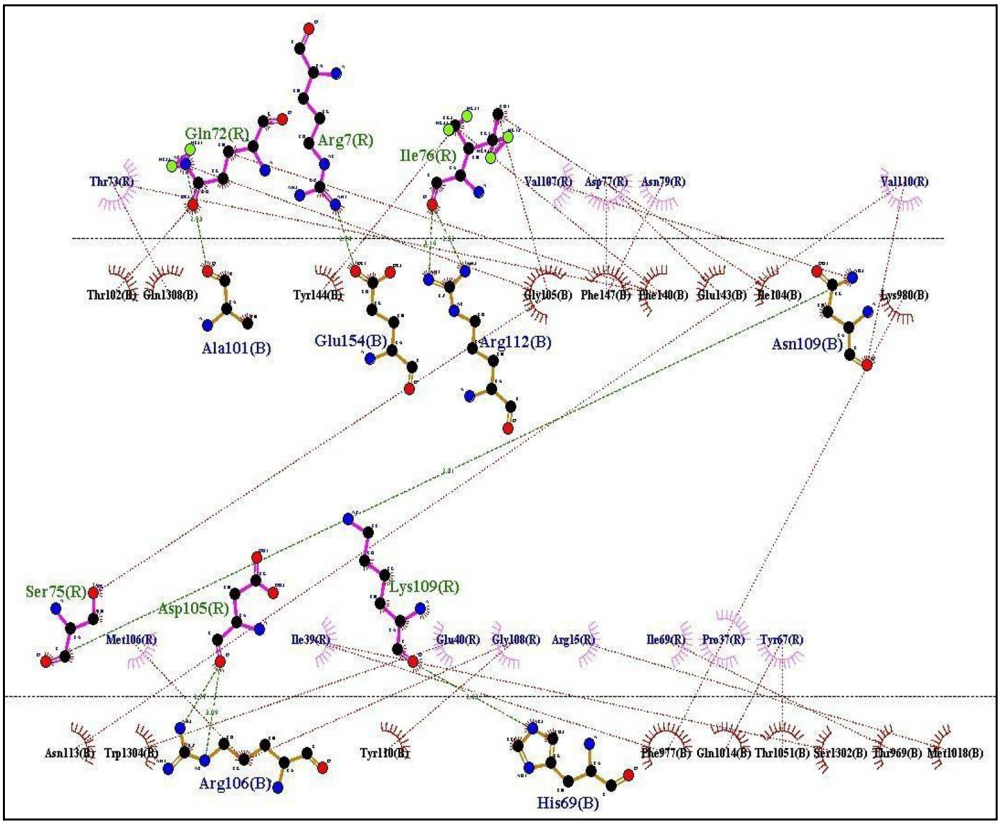

Fig.-S1: The mTOR kinase and RHEB GTPase interface: The ligplot diagram shows residues *His69, Ala101, Arg106, Asn109, Arg112* and *Glu 154* from the N-Heat domain of the mTOR kinase are involved in the Hydrogen bond (Green) formation while the residues *Arg7, Gln72, Ser75, Ile76, Asp105* and *Lys109* are from the RHEB. The rest all interacting residues of the mTOR are in brown colour while that of RHEB are in Pink. Labellings: - Red: Hydrophobic residues from RHEB, Pink: Hydrophobic residues from N-Heat, Green Bonds: Hydrogen bonds, Red Bonds: Interaction other than H-Bonds.

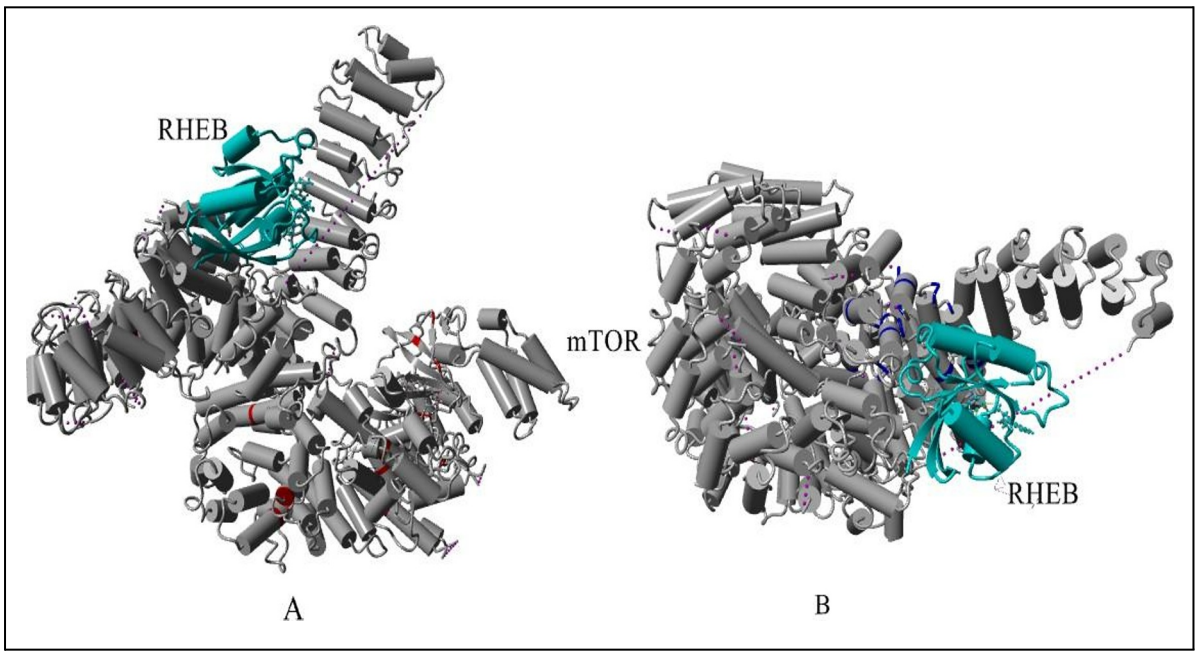

Fig S2: The positions of the retrieved mutations and the "evolutionary changes identified" in the mTOR kinase sequence. Colour code - Red: Mutations, Blue: Evolutionary changes, Gray: mTOR kinase and Cyane: RHEB.

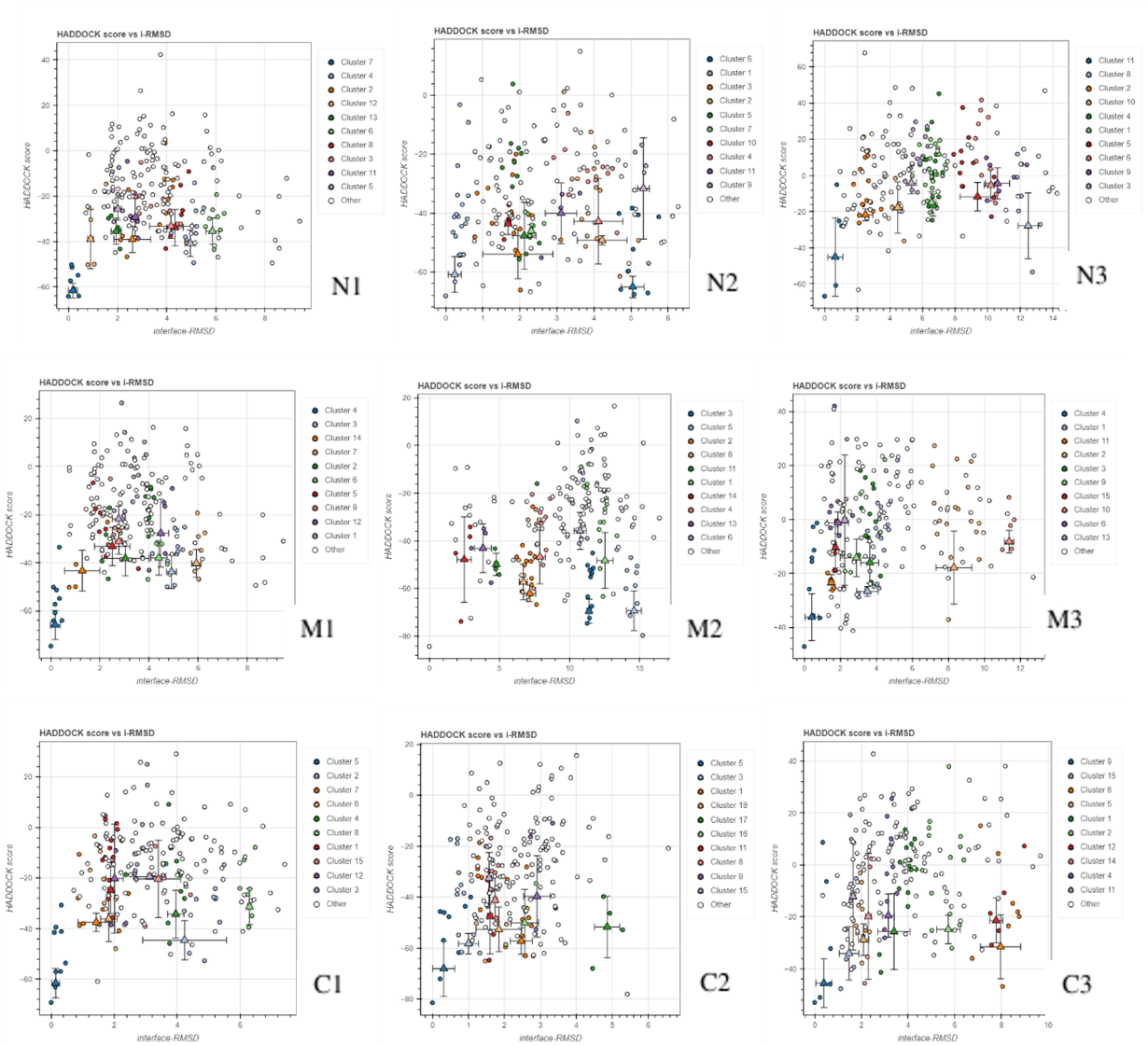

Fig-S3: HADDOCK docking results of designed peptides with RHEB GTPase. Scatter plots showing HADDOCK score versus interface-RMSD (i-RMSD) for all nine designed peptides: native peptides (N1, N2, N3), modified peptides (M1, M2, M3), and constructive peptides (C1, C2, C3). Each point represents a docking pose, with distinct clusters color-coded according to the legend in each panel. Lower HADDOCK scores (more negative) indicate stronger predicted binding affinity, while lower i-RMSD values (<2.0 Å) represent structurally convergent and reliable binding modes. Peptides M2 and M3 exhibit the most favorable profiles with low scores and low i-RMSD, followed by C3 and M1. Native peptides generally show weaker binding predictions. These results support the potential of M2, M3, and C3 as candidate inhibitors of mTOR-RHEB interaction.

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