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Targeting of integrin αvβ3 with different sequence of RGD peptides: A molecular dynamics simulation study

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Abstract. Integrin αvβ3 is one of the receptors expressed in cancer cells. RGD peptides have the potential to target integrin αvβ3 (receptor), which can increase drug delivery efficiency. In this study, 55 different RGD dimer motifs were investigated. At first, the binding energy between RGD peptides and the receptor was calculated using molecular docking. Then, three RGD peptides with the strongest binding energy with the receptor were selected, and their dynamic adsorption on the receptor was simulated by molecular dynamics (MD). The obtained results showed that a sequence that has RGD at the beginning and end with tryptophan (TRP) has strong Lennard-Jones (LJ) and electrostatic interactions with Integrin αvβ3 and has changed the conformation of receptor significantly, which analyzed by root mean square deviation (RMSD) and radius of gyration.
Keywords: integrin αvβ3 receptor; molecular dynamics (MD) simulation; RGD peptide; RMSD; targeting

1. Introduction

Selective action is one of the essential aspects of designing anticancer drugs. Current drugs should be not only capable of selectively targeting and killing cancerous cells but also be able to distinguish between normal and cancerous cells. Up to now, attempts have been made to design particular drugs to target a specific site found only in cancerous cells (Plantefaber and Hynes 1989, Giancotti and Ruoslahti 1999, Zitzmann *et al*. 2002, Bardania *et al*. 2019, Akrami *et al*. 2021, Mahmoudi *et al*. 2021). Macro-molecules such as receptors overexpressed in cancerous cells are mostly considered specific targets. We know that integrin receptors are expressed in both normal and cancerous cells. However, it was observed that certain types of integrins have significantly overexpressed in some cancers. For example, αvβ3 integrin is highly expressed in various tumors, such as bone tumors, glioblastomas, neuroblastomas, and lung and breast cancers (Zitzmann *et al*. 2002, Kwakwa and Sterling 2017, Krishn *et al*. 2019). Therefore, integrin receptors could be used as selective targets for developing specific cancer diagnoses and treatments. Integrin receptors composed of αβ heterodimer are responsible for intercellular adhesion and adhesion between the extracellular matrix and the cell (Plantefaber and Hynes 1989, Chan *et al*. 1991, Giancotti and Ruoslahti 1999, Zitzmann *et al*. 2002, Mezu-Ndubuisi and Maheshwari 2020). Integrins can provide bi-directional transmembrane signals through interaction with cellular surfaces or extracellular matrix, affecting adhesion, proliferation, differentiation, growth, migration, and cell survival (Zitzmann *et al*. 2002, Cheah and Andrews 2018, Shimaoka *et al*. 2019). αIIbβ3, αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, α5β1 and α8β1

Integrin receptors, for instance, αIIbβ3, αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, α5β1, and α8β1 have high affinity to interact with RGD (arginine-glycine-aspartic acid) motif in natural proteins for example fibronectin, vitronectin, fibrinogen, and Osteopontin. Numerous studies have revealed that integrins with high affinity to interact with the RGD motif are highly expressed in malignant cells (Plantefaber and Hynes 1989, Chan *et al*. 1991, Nieberler *et al*. 2017). As an illustration, cells with high metastasis potential showed a significant increase in αvβ3 integrin expression compared to cells with low metastasis potential (Albelda *et al*. 1990, Gehlsen *et al*. 1992, Tang *et al*. 2020). Based on these findings, the high expression of RGD-binding integrins in cancerous cells may be a specific sign of the presence of specific tumors (Albelda *et al*. 1990, Gehlsen *et al*. 1992, Goligorsky *et al*. 1998, Aksorn and Chanvorachote 2019, Mahmoudi *et al*. 2021). It is indicated that RGD-based cationic polymers and lipids efficiently target cancerous cells (Fu *et al*. 2019).

In addition, the crystal structure determination of the integrin receptors showed the presence of RGD tripeptide binding to the receptor (Xiong *et al*. 2002, Xiao *et al*. 2004, Nagae *et al*. 2012). Numerous RGD-containing peptides with various origins have been identified with high affinity to selectively bind to integrin receptors overexpressed in cancerous cells. However, RGD peptide-based drug therapy with high efficiency for eliminating human tumor cells has yet to be commercially available (Marchini *et al*. 2012, Fanelli *et al*. 2014, Panzeri *et al*. 2015, Toum *et al*. 2015). It

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is indicated that RGDVF (arginine-glycine-aspartate-phenylalanine-valine) in cyclic form has strong interaction with the integrin receptor. Also, the optimum molar ratio of RGD peptide to integrin receptor is 2:1 (Yu *et al*. 2014).

Moreover, it has been confirmed that replacing only one amino acid in the RGD tripeptide weakens its ability to bind to the αvβ3 integrin (Xiong *et al*. 2002). Based on structural studies performed on ligand-receptor complexes, it has been determined that the exchange of aspartic residue with glutamate causes a spatial barrier, which in turn prevents the formation of chemical bonds for the stability of the complex (Gurrath *et al*. 1992, Jwad *et al*. 2020). In accordance with structural studies to determine the structure of peptides containing RGD motif with high affinity for the receptor (Kok *et al*. 2002, Wu *et al*. 2017), it was found that cyclic polymeric forms of RGD have the highest tendency to bind to integrin receptors than linear forms (Yu *et al*. 2014). Previous studies investigated the short sequences of RGD peptides (Yu *et al*. 2014, Fu *et al*. 2019). In contrast, the peptide conformation needs to have a proper 2D structure to effectively interact with RGD-containing peptides with integrin receptors (Saudek *et al*. 1991, Wakefield *et al*. 2015). In the case of the polymeric forms, many linear peptide units increase the concentration of ligand regions near the receptor. It causes multiple ligand interactions with the receptor. This event is one of the advantages of the polymeric form over the monomeric form of peptides containing the RGD motif (Didier *et al*.). Furthermore, previous studies have shown that RGD dimers have a higher affinity than monomers (Yu *et al*. 2014, Sacco *et al*. 2019). Therefore, in this study, the interaction of 55 different RGD dimer motifs with αvβ3 integrin was investigated by molecular docking and molecular dynamics (MD) simulation. The investigated peptides have 13 amino acids and also have a secondary structure, which is the innovation of this study in comparison with previous studies (Leng and Mixson 2005, Leng *et al*. 2005, Yu *et al*. 2014).

2. Model and method

2.1 The structural models

The crystal structure of the extracellular segment of integrin αvβ3 (receptor) (PDB ID=1L5G) was obtained from the protein data bank (Rose, Beran *et al*. 2010). To investigate the effect of RGD peptide sequence on interaction with the receptor, 55 different sequences of RGD peptide were generated by the I-TASSER server (Yang *et al*. 2015), which has 13 amino acids. Their sequences are shown in Table 1 in Supporting Information.

2.2 Method

In this study, the binding energy between 55 RGD peptides and the receptor was obtained with molecular docking. Then, the RGD peptides with the strongest binding energy with the receptor were selected, and their adsorption on the receptor was simulated by the molecular dynamics method.

All of the MD simulations were performed by GROMACS 5.1.4 software package (Van Der Spoel *et al*. 2005). At first, equilibrated receptor and RGD peptide structures should be obtained for docking. For this purpose, each of the RGD peptides and receptors was simulated separately in pH=5.5 according to the condition of the cancer cell. This simulation was performed for 25 ns at a temperature of 310 K and 1 bar pressure. The structure of the receptor (1L5G (Xiong *et al*. 2002)) is in the state where the RGD is adsorbed on it. Based on the RGD adsorption site on receptor, the docking box was selected, which is shown in Fig. 1. The grid box of dimension 36 Å × 26 Å × 26 Å with grid spacing 1 Å (center 15,50,60) was used for docking.

The binding energy between receptor and 55 RGD peptides was calculated using ClusPro server (Comeau *et al*. 2004, Kozakov *et al*. 2017) where represents the interaction energy between two proteins using an expression of the form $E = w1E_{rep} + w2E_{attr} + w3E_{elec} + w4E_{DARS}$, where E_{rep} and E_{attr} denote the repulsive and attractive contributions to the van der Waals interaction energy, and E_{elec} is an electrostatic energy term. E_{DARS} is a pairwise structure-based potential constructed by the Decoys as the Reference State (DARS) approach (Lorenzen and Zhang 2007), and it primarily represents desolvation contributions, i.e., the free energy change due to the removal of the water molecules from the interface (Comeau *et al*. 2004). The coefficients w1, w2, w3, and w4 define the weights of the corresponding terms and are optimally selected for different types of docking problems. The coefficients of w1, w2, w3, and w4 are 0.4, -0.4, 600 and 1 in this study. Docking was performed with Fast Fourier Transform (FFT) method with a flexible receptor (Porter *et al*. 2017). The ClusPro server gives a score for each RGD peptide based on the interaction energy with the receptor. A first approximation of interaction between RGD peptides and receptor was obtained by using docking results and then, three RGD peptides that have strong interactions with receptor were selected for molecular dynamics (MD) simulation.

According to the docking results, three peptides with the strongest interaction with the receptor were selected, and their adsorption on the receptor was simulated by the coarse-grained molecular dynamics simulation method. The MARTINI coarse-grained force field (CGFF) (Marrink *et al*. 2007, Monticelli *et al*. 2008), was used to calculate the beads interactions where almost four heavy atoms are represented as a single bead. A cutoff radius of 1.2 nm was applied for electrostatic and van der Waals interactions. The particle mesh Ewald (PME) (Essmann *et al*. 1995) summation method was utilized for electrostatic interactions. The periodic boundary conditions (PBC) were applied in the simulation box's x, y, and z directions. The neighbor searching update frequency was set to 10 steps. The time step of all simulations was set to 20 fs. By utilizing the Berendsen (Berendsen *et al*. 1984) coupling method, the temperature and pressure of the simulation boxes were fixed at 310 K and 1 bar, respectively. Initial velocities were randomly generated from the Maxwell-Boltzmann distribution at the desired temperature. Visual

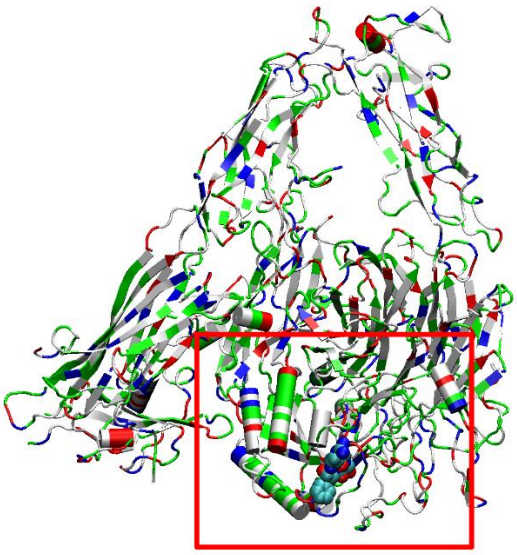

Fig. 1 The schematic of integrin αvβ3 receptor and the adsorption site of RGD. The white, green, red and blue colors in peptide structure represent nonpolar, polar, acidic and basic amino acids, respectively (Xiong *et al*. 2002)

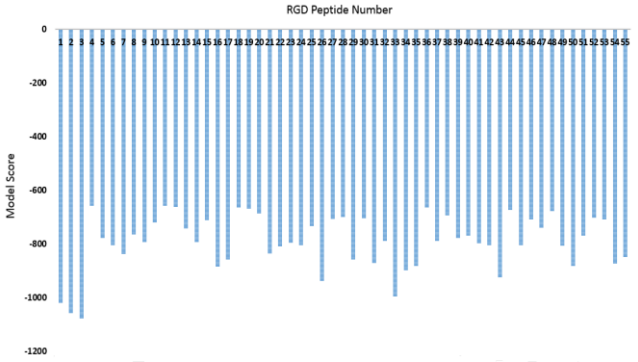

Fig. 2 The model scores of RGD peptides with receptor

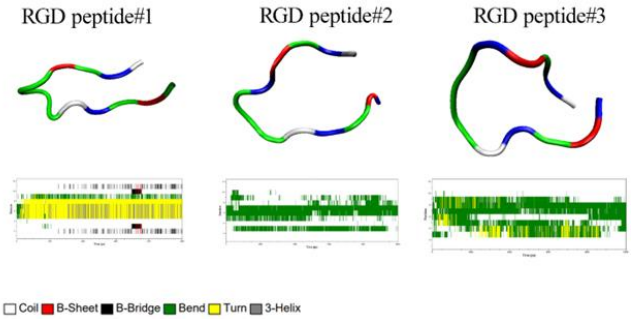

Fig. 3 The optimized structures of RGD peptides 1,2 and 3 used for docking and their secondary structures. The white, green, red and blue colors in peptide structure represent nonpolar, polar, acidic and basic amino acids, respectively

molecular dynamics (VMD 1.9.3) (Humphrey *et al*. 1996) was used to visualize the molecules, and all the analyses were performed by GROMACS software. For investigating the adsorption of peptides on the receptor, first, the receptor was placed at the center of the simulation box (11×11×11 nm^3), and then three RGD peptides were randomly

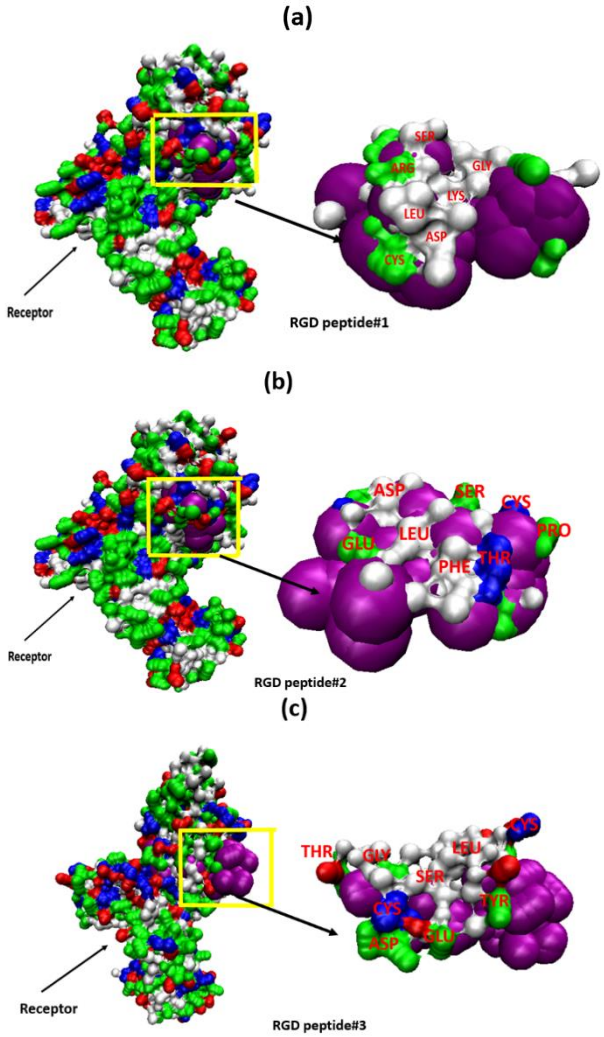

Fig. 5 The snapshots of final step of simulation for interaction of receptor with (a) RGD peptide#1, (b) RGD peptide#2 and (c) RGD peptide#3. The violet vdW spheres show RGD peptides. The receptor is shown with white, green, red and blue surface which represent nonpolar, polar, acidic and basic amino acids, respectively

distributed around the receptor. The NVT simulation for 10 ns was performed, and the temperature was set at natural body temperature. The pressure was adjusted at atmospheric pressure in the NPT ensemble for 10 ns. Then the MD step was performed for 100 ns for data collection, and the last 30 ns were used for analysis. Also, to ensure the adsorption sites of the peptides, the simulations were repeated once.

3. Results and discussion

3.1 Docking results

The model score of each RGD peptide which is a criterion of interaction energy with the receptor is illustrated in Fig. 2. The represented results in Fig. 2 demonstrate that RGD peptides#1,2 and 3 have strong interaction energy with the receptor between 55 peptides and are selected for

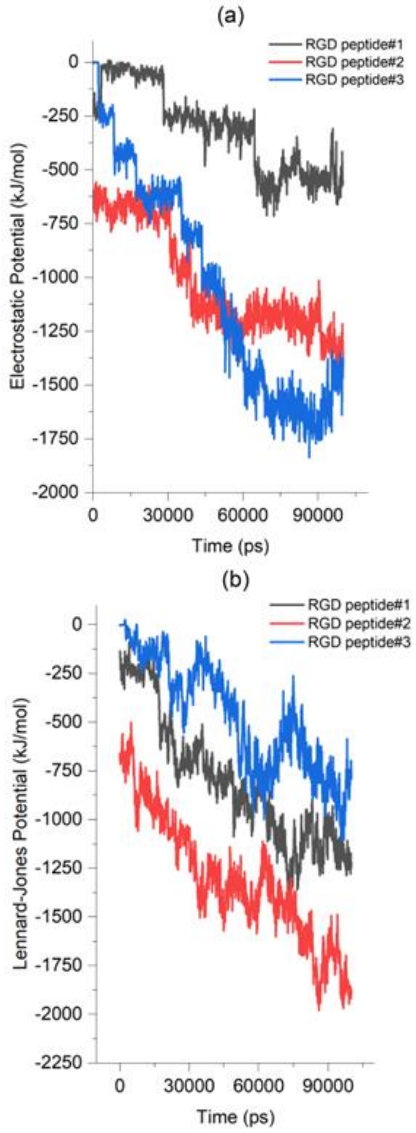

Fig. 6 (a) electrostatic potential and (b) Lennard-Jones between RGD peptides and receptor versus simulation time

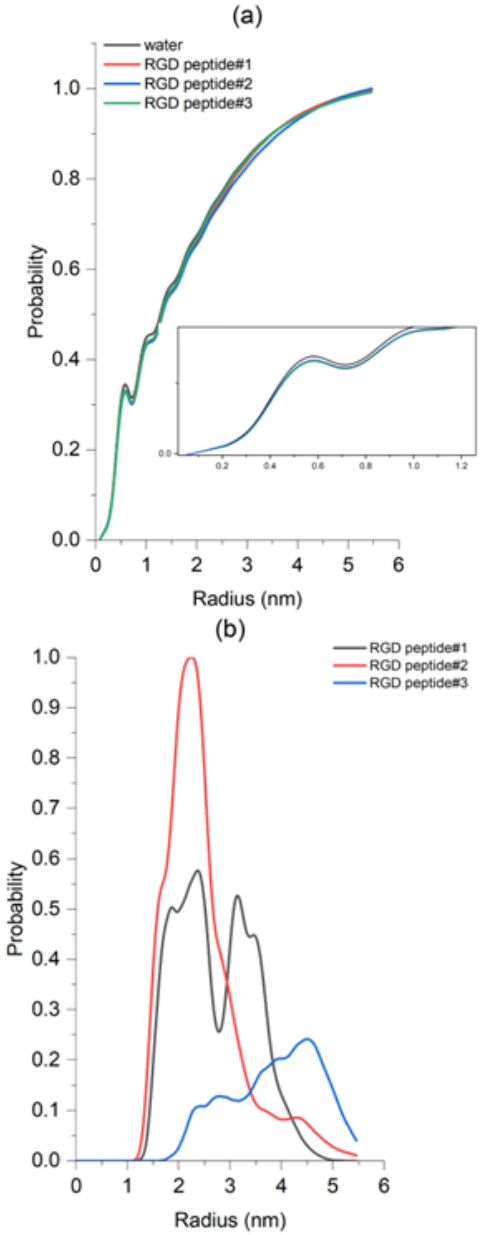

Fig. 7 Radial probability of finding (a) water molecules and (b) RGD peptides around the receptor

MD simulation. The structure of the peptides used for docking and their secondary structure are shown in Fig. 3. The helical wheel plots of RGD peptides# 1, 2 and 3 are illustrated in Fig. S1. RGD peptides 1,2 and 3 are different in first, fifth, ninth and thirteenth amino acids and also, they have RGD at the beginning and end of the sequence. Threonine (THR), tryptophan (TRP) and leucine (LEU) are different amino acids in RGD peptides# 1,2 and 3, respectively.

3.2 Interaction potentials

The initial and final snapshots of the simulation box for the adsorption of RGD peptides on the receptor are illustrated in Fig. 4. Initially, the RGD peptides are randomly distributed around the receptor in the simulation box. The RGD peptides are adsorbed during the simulation on the receptor due to Lennard-Jones (LJ) and electrostatic interactions. Fig. 4 illustrates that in the simulation of RGD peptide 1, at first, three peptides are randomly distributed around the receptor. While at the end of simulation time, two peptides are adsorbed on different sites of the receptor, and one peptide does not adsorb. In the simulation of RGD peptide 2, two of three RGD peptides are adsorbed on the same receptor sites. The obtained results from a simulation of RGD peptide 3 indicated that one of the three RGD peptide 3 is absorbed on the extracellular region of the receptor. These results elucidate that the optimum ratio between peptide and receptor is 2:1, as it is shown in previous studies (Yu *et al*. 2014, Fu *et al*. 2019). Adsorption of all three inserted peptides on the receptor was observed only for RGD peptide 2, which elucidates a strong interaction between them.

The adsorption sites of RGD peptides on the receptor are illustrated in Fig. 5. Fig. 5(a) indicates that the adsorption site of RGD peptide1 on the receptor comprises

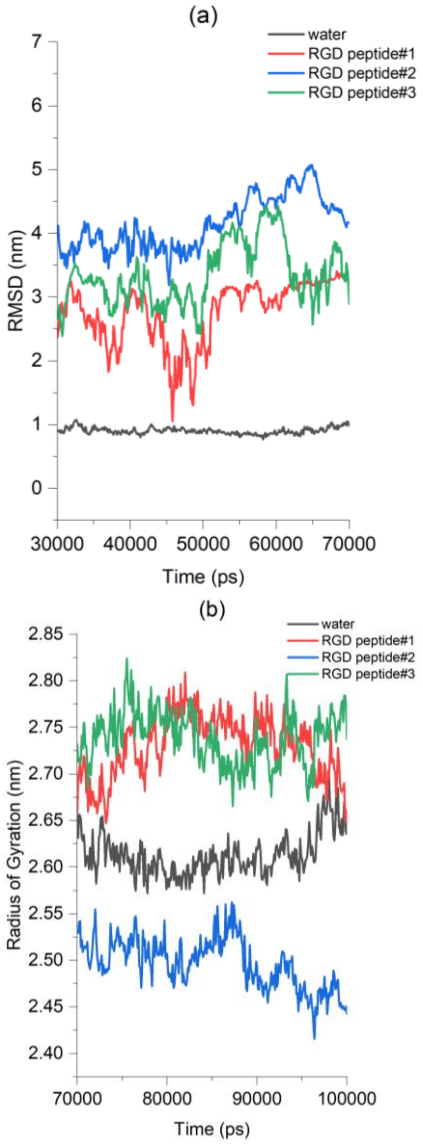

Fig. 8 (a) The root mean square deviation (RMSD) and (b) radius of gyration of the receptor in water medium and after RGD peptides adsorption

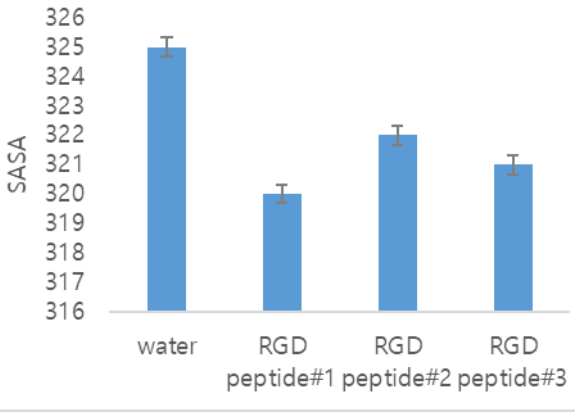

Fig. 9 The solvent accessible surface area (SASA) of the receptor in water medium and after RGD peptides adsorption

polar and non-polar amino acids. Figs. 5(b) and 5(c) demonstrate that RGD peptides 2 and 3 with 2 positive charges have been adsorbed on aspartic acid (ASP) and glutamic acid (GLU) which are negatively charged amino acids.

The binding energy between RGD peptides and the receptor is due to Lennard-Jones (LJ) and electrostatic potentials. The LJ potentials between RGD peptides and the receptor are shown in Fig. 5(b). As it is seen in this figure, at first, RGD peptides are distributed randomly around the receptor in simulation box and the LJ potentials are weak. As the simulation proceeds, the distance between the RGD peptides and the receptor decreases and the LJ potentials approaches to an equilibrium value. The negative values of LJ potentials reveal attraction energy between RGD peptides and the receptors. Decreasing fluctuation of the curves in Fig. 5 in the last 70 ns of simulation time confirms the equilibration of systems. The electrostatic potentials between RGD peptides and receptor are represented in Fig. 5(a). It can be seen clearly in Fig. 5(a) that RGD peptides 2 and 3 with positive charge have strong electrostatic potential with receptor which has a negative charge. While RGD peptide 1 with zero charge has weak electrostatic interaction with the receptor. These obtained values for LJ and electrostatic potentials is in accordance with previous studies (Leng and Mixson 2005, Leng *et al*. 2005, Yu *et al*. 2014).

The radial probabilities of finding RGD peptides around the receptor have been calculated and plotted in Fig. 7(b). The highest peak of RGD peptide 2 in Fig. 5(a) confirms strong interactions between RGD peptide 2 and receptor which were seen in Fig. 6. Considering the number of contacts between RGD peptides and receptor in Fig. S2 proves the strong interaction between RGD peptide 2 and receptor. Two peaks in RDF plot of RGD peptide 1, confirms two different adsorption sites of peptides on the receptor. The peak of RGD peptide 3 was observed at a distance of 4.5 nm from the center of mass of the receptor which confirms the weak interaction potential between RGD peptide 3 and the receptor as was observed in Fig. 4.

3.3 Conformational changes

The root means square deviation (RMSD) of the receptor after RDG peptide adsorption has been calculated and depicted in Fig. 8(a). It can be seen in Figure 8a that the conformational changes of the receptor after adsorption of RGD peptide 2 are remarkable, where the reason for this behavior is the strong interaction potentials that were discussed before. The radius of gyration of the receptor after the adsorption of RGD peptides is represented in Fig. 8(b). Comparison of the radius of gyration of receptor in a water medium and after RGD peptide adsorption confirms the significant conformational changes of the receptor after the adsorption of RGD peptide 2.

3.4 Hydrophilicity

The conformational changes of the receptor after adsorption of RGD peptides alter the hydrophilicity of the

receptor. The radial probability of finding water molecules around the receptor in water medium and after drug adsorption is represented in Fig. 7(a). As it is seen in Fig. 7(a), each peak shows a layer of water around the receptor and the height of peak in water medium is higher than after adsorption of RDG peptides. As it is illustrated in Fig. 9, the solvent accessible surface area (SASA) of the receptor has decreased from 325 to 320 nm2 after adsorption of RGD peptides which confirms conformational changes of the receptors as discussed in section 3.3.

4. Conclusions

Integrin αvβ3 is a transmembrane-spanning receptor in cancer cells. The most common integrins recognize the tripeptide sequence Arg-Gly-Asp (RGD), found in many extracellular matrix adhesive proteins. In This study, the adsorption of different RGD peptide sequences integrin αvβ3 was investigated. 55 different sequences of RGD peptides with 13 Amino acids were considered. The selected peptides have a secondary structure that was not considered in previous studies. At first, by using molecular docking, the binding energy between RGD peptides and the receptor was examined. Then, three RGD peptides with high binding energy with the receptor were selected for MD simulation. The conformational changes of the receptor after RGD adsorption were analyzed by RMSD, RDF, the radius of gyration, and SASA. The obtained results demonstrated that RGD peptides that have two RGD at the beginning and end of their sequence have strong interaction with the receptor, and also RGD peptides that have tryptophan (TRP) have strong LJ and electrostatic interactions with the receptor. This optimum sequence is effective for designing drug delivery carriers for cancer cells which increases the efficiency of targeting cancer cells.

References

Akrami, M., Samimi, S., Alipour, M., Bardania, H., Ramezanpour, S., Najafi, N., Hosseinkhani, S., Kamankesh, M., Haririan, I. and Hassanshahi, F. (2021), "Potential anticancer activity of a new pro-apoptotic peptide–thioctic acid gold nanoparticle platform", *Nanotechnology*, **32**(14), 145101. https://doi.org/10.1088/1361-6528/abd3cb

Aksorn, N. and Chanvorachote, P. (2019), "Integrin as a molecular target for anti-cancer approaches in lung cancer", *Anticancer Res.*, **39**(2), 541-548. https://doi.org/10.21873/anticanres.13146.

Albelda, S.M., Mette, S.A., Elder, D.E., Stewart, R., Damjanovich, L., Herlyn, M. and Buck, C.A. (1990), "Integrin distribution in malignant melanoma: Association of the β3 subunit with tumor progression", *Cancer Res.*, **50**(20), 6757-6764.

Bardania, H., Shojaosadati, S.A., Kobarfard, F., Morshedi, D., Aliakbari, F., Tahoori, M.T. and Roshani, E. (2019), "RGD-modified nano-liposomes encapsulated eptifibatide with proper hemocompatibility and cytotoxicity effect", *Iranian J. Biotechnol.*, **17**(2), e2008. https://doi.org/10.21859/ijb.2008

Berendsen, H.J., Postma, J.V., van Gunsteren, W.F., DiNola, A. and Haak, J.R. (1984), "Molecular dynamics with coupling to an external bath", *J. Chem. Phys.*, **81**(8), 3684-3690. https://doi.org/10.1063/1.448118

Chan, B., Matsuura, N., Takada, Y., Zetter, B.R. and Hemler, M.E. (1991), "In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells", *Science*, **251**(5001), 1600-1602.

Cheah, M. and Andrews, M.R. (2018), "Integrin activation: implications for axon regeneration", *Cells*, **7**(3), 20. https://doi.org/10.1126/science.2011740.

Comeau, S.R., Gatchell, D.W., Vajda, S. and Camacho, C.J. (2004), "ClusPro: A32en automated docking and discrimination method for the prediction of protein complexes", *Bioinform.*, **20**(1), 45-50. https://doi.org/10.1093/bioinformatics/btg371

Didier, B., Catherine, S., Alexeï, G., Pascal, D. and Jean-Luc, C. "Clustering and internalization of integrin alphavbeta3 with a tetrameric RGD-synthetic peptide",

Essmann, U., Perera, L., Berkowitz, M.L., Darden, T., Lee, H. and Pedersen, L.G. (1995), "A smooth particle mesh Ewald method", *J. Chem. Phys.*, **103**(19), 8577-8593. https://doi.org/10.1063/1.470117

Fanelli, R., Schembri, L., Piarulli, U., Pinoli, M., Rasini, E., Paolillo, M., Galiazzo, M.C., Cosentino, M. and Marino, F. (2014), "Effects of a novel cyclic RGD peptidomimetic on cell proliferation, migration and angiogenic activity in human endothelial cells", *Vascular Cell*, **6**(1), 1-9. https://doi.org/10.1186/2045-824X-6-11

Fu, S., Xu, X., Ma, Y., Zhang, S. and Zhang, S. (2019), "RGD peptide-based non-viral gene delivery vectors targeting integrin αvβ3 for cancer therapy", **27**(1), 1-11. https://doi.org/10.1080/1061186X.2018.1455841

Gehlsen, K.R., Davis, G.E. and Sriramarao, P. (1992), "Integrin expression in human melanoma cells with differing invasive and metastatic properties", *Clin. Exp. Metastas.*, **10**(2), 111-120. https://doi.org/10.1007/BF00114587

Giancotti, F.G. and Ruoslahti, E. (1999), "Integrin signaling", *Science*, **285**(5430), 1028-1033. https://doi.org/10.1126/science.285.5430.1028

Goligorsky, M.S., Kessler, H. and Romanov, V.I. (1998), "Molecular mimicry of integrin ligation: therapeutic potential of arginine-glycine-aspartic acid (RGD) peptides", *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association*, **13**(2), 254-263.

Gurrath, M., Müller, G., Kessler, H., Aumailley, M. and Timpl, R. (1992), "Conformation/activity studies of rationally designed potent anti-adhesive RGD peptides", *Eur. J. Biochem.*, **210**(3), 911-921. https://doi.org/10.1111/j.1432-1033.1992.tb17495.x

Humphrey, W., Dalke, A. and Schulten, K. (1996), "VMD: visual molecular dynamics", *J. Mol. Graph.*, **14**(1), 33-38. https://doi.org/10.1016/0263-7855(96)00018-5

Jwad, R., Weissberger, D. and Hunter, L. (2020), "Strategies for fine-tuning the conformations of cyclic peptides", *Chem. Rev.*, **120**(17), 9743-9789. https://doi.org/10.1021/acs.chemrev.0c00013

Kok, R.J., Schraa, A.J., Bos, E.J., Moorlag, H.E., Ásgeirsdóttir, S.A., Everts, M., Meijer, D.K. and Molema, G. (2002), "Preparation and functional evaluation of RGD-modified proteins as αvβ3 integrin directed therapeutics", *Bioconjugate Chem.*, **13**(1), 128-135. https://doi.org/10.1021/bc015561+

Kozakov, D., Hall, D.R., Xia, B., Porter, K.A., Padhorny, D., Yueh, C., Beglov, D. and Vajda, S. (2017), "The ClusPro web server for protein–protein docking", *Nature Protoc.*, **12**(2), 255-278. https://doi.org/10.1038/nprot.2016.169

Krishn, S.R., Singh, A., Bowler, N., Duffy, A.N., Friedman, A., Fedele, C., Kurtoglu, S., Tripathi, S.K., Wang, K. and Hawkins, A. (2019), "Prostate cancer sheds the αvβ3 integrin in vivo through exosomes", *Matrix Biol.*, **77**, 41-57. https://doi.org/10.1016/j.matbio.2018.08.004

Kwakwa, K.A. and Sterling, J.A. (2017), "Integrin αvβ3 signaling

in tumor-induced bone disease", *Cancers*, **9**(7), 84. https://doi.org/10.3390/cancers9070084

Leng, Q. and Mixson, A.J. (2005), "Modified branched peptides with a histidine-rich tail enhance in vitro gene transfection", **33**(4), e40-e40. https://doi.org/10.1093/nar/gni040

Leng, Q., Scaria, P., Zhu, J., Ambulos, N., Campbell, P. and Mixson, A.J. (2005), "Highly branched HK peptides are effective carriers of siRNA", **7**(7), 977-986. https://doi.org/10.1002/jgm.748

Lorenzen, S. and Zhang, Y. (2007), "Identification of near-native structures by clustering protein docking conformations", *Proteins Struct. Funct. Bioinform.*, **68**(1), 187-194. https://doi.org/10.1002/prot.21442

Mahmoudi, R., Ashraf Mirahmadi-Babaheidri, S., Delaviz, H., Fouani, M.H., Alipour, M., Jafari Barmak, M., Christiansen, G. and Bardania, H. (2021), "RGD peptide-mediated liposomal curcumin targeted delivery to breast cancer cells", *J. Biomater. Appl.*, **35**(7), 743-753. https://doi.org/10.1177/0885328220949367

Marchini, M., Mingozzi, M., Colombo, R., Guzzetti, I., Belvisi, L., Vasile, F., Potenza, D., Piarulli, U., Arosio, D. and Gennari, C. (2012), "Cyclic RGD peptidomimetics containing bifunctional diketopiperazine scaffolds as new potent integrin ligands", *Chem. A Eur. J.*, **18**(20), 6195-6207. https://doi.org/10.1002/chem.201200457

Marrink, S.J., Risselada, H.J., Yefimov, S., Tieleman, D.P. and De Vries, A.H. (2007), "The MARTINI force field: Coarse grained model for biomolecular simulations", *J. Phys. Chem. B*, **111**(27), 7812-7824. https://doi.org/10.1021/jp071097f

Mezu-Ndubuisi, O.J. and Maheshwari, A. (2020), "The role of integrins in inflammation and angiogenesis", *Pediatric Res.*, 1-8. https://doi.org/10.1038/s41390-020-01177-9

Monticelli, L., Kandasamy, S.K., Periole, X., Larson, R.G., Tieleman, D.P. and Marrink, S.J. (2008), "The MARTINI coarse-grained force field: Extension to proteins", *J. Chem. Theor. Comput.*, **4**(5), 819-834. https://doi.org/10.1021/ct700324xr

Nagae, M., Re, S., Mihara, E., Nogi, T., Sugita, Y. and Takagi, J. (2012), "Crystal structure of α5β1 integrin ectodomain: Atomic details of the fibronectin receptor", *J. Cell Biol.*, **197**(1), 131-140. https://doi.org/10.1083/jcb.201111077

Nieberler, M., Reuning, U., Reichart, F., Notni, J., Wester, H.J., Schwaiger, M., Weinmüller, M., Räder, A., Steiger, K. and Kessler, H. (2017), "Exploring the role of RGD-recognizing integrins in cancer", *Cancers*, **9**(9), 116. https://doi.org/10.3390/cancers9090116

Panzeri, S., Zanella, S., Arosio, D., Vahdati, L., Dal Corso, A., Pignataro, L., Paolillo, M., Schinelli, S., Belvisi, L. and Gennari, C. (2015), "Cyclic isoDGR and RGD peptidomimetics containing bifunctional diketopiperazine scaffolds are integrin antagonists", *Chem. A Eur. J.*, **21**(16), 6265-6271. https://doi.org/10.1002/chem.201406567

Plantefaber, L.C. and Hynes, R.O. (1989), "Changes in integrin receptors on oncogenically transformed cells", *Cell*, **56**(2), 281-290. https://doi.org/10.1016/0092-8674(89)90902-1

Porter, K.A., Xia, B., Beglov, D., Bohnuud, T., Alam, N., Schueler-Furman, O. and Kozakov, D.J.B. (2017), "ClusPro PeptiDock: Efficient global docking of peptide recognition motifs using FFT", **33**(20), 3299-3301. https://doi.org/10.1093/bioinformatics/btx216

Rose, P.W., Beran, B., Bi, C., Bluhm, W.F., Dimitropoulos, D., Goodsell, D.S., Prlić, A., Quesada, M., Quinn, G.B. and Westbrook, J.D. (2010), "The RCSB Protein Data Bank: redesigned web site and web services", *Nucleic Acids Res.*, **39**(suppl 1), D392-D401. https://doi.org/10.1093/nar/gkq1021

Sacco, G., Dal Corso, A., Arosio, D., Belvisi, L., Paolillo, M., Pignataro, L. and Gennari, C. (2019), "A dimeric bicyclic RGD ligand displays enhanced integrin binding affinity and strong biological effects on U-373 MG glioblastoma cells", *Organ. Biomol. Chem.*, **17**(39), 8913-8917. https://doi.org/10.1039/C9OB01811E

Saudek, V., Atkinson, R.A. and Pelton, J.T. (1991), "Three-dimensional structure of echistatin, the smallest active RGD protein", *Biochem.*, **30**(30), 7369-7372. https://doi.org/10.1021/bi00244a003

Shimaoka, M., Kawamoto, E., Gaowa, A., Okamoto, T. and Park, E.J. (2019), "Connexins and integrins in exosomes", *Cancers*, **11**(1), 106. https://doi.org/10.3390/cancers11010106

Tang, L., Xu, M., Zhang, L., Qu, L. and Liu, X. (2020), "Role of αVβ3 in prostate cancer: Metastasis initiator and important therapeutic target", *OncoTargets Therapy*. **13** 7411.

Toum, V., Bolley, J., Lalatonne, Y., Barbey, C., Motte, L., Lecouvey, M., Royer, J., Dupont, N. and Pérard-Viret, J. (2015), "In silico studies, synthesis and binding evaluation of substituted 2-pyrrolidinones as peptidomimetics of RGD tripeptide sequence", *Eur. J. Med. Chem.*, **93**, 360-372. https://doi.org/10.1016/j.ejmech.2015.02.017

Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E. and Berendsen, H.J. (2005), "GROMACS: Fast, flexible, and free", *J. Comput. Chem.*, **26**(16), 1701-1718. https://doi.org/10.1002/jcc.20291. PMID: 16211538.

Wakefield, A.E., Wuest, W.M. and Voelz, V.A. (2015), "Molecular simulation of conformational pre-organization in cyclic RGD peptides", *J. Chem. Inform. Model.*, **55**(4), 806-813. https://doi.org/10.1021/ci500768u

Wu, Z., Cheng, X., Hong, H., Zhao, X. and Zhou, Z. (2017), "New potent and selective αvβ3 integrin ligands: Macrocyclic peptides containing RGD motif synthesized by sortase A-mediated ligation", *Bioorgan. Med. Chem. Lett.*, **27**(9), 1911-1913. https://doi.org/10.1016/j.bmcl.2017.03.035

Xiao, T., Takagi, J., Coller, B.S., Wang, J.H. and Springer, T.A. (2004), "Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics", *Nature*, **432**(7013), 59-67. https://doi.org/10.1038/nature02976

Xiong, J.-P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S.L. and Arnaout, M.A. (2002), "Crystal structure of the extracellular segment of integrin αVβ3 in complex with an Arg-Gly-Asp ligand", *Science*, **296**(5565), 151-155. https://doi.org/10.1126/science.1069040

Xiong, J.-P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S.L. and Arnaout, M.A.J.S. (2002), "Crystal structure of the extracellular segment of integrin αVβ3 in complex with an Arg-Gly-Asp ligand", **296**(5565), 151-155. https://doi.org/10.1126/science.1069040

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J. and Zhang, Y. (2015), "The I-TASSER Suite: protein structure and function prediction", *Nature Methods*, **12**(1), 7-8. https://doi.org/10.1038/nmeth.3213

Yu, Y.P., Wang, Q., Liu, Y.C. and Xie, Y. (2014), "Molecular basis for the targeted binding of RGD-containing peptide to integrin αVβ3", *Biomater.*, **35**(5), 1667-1675. https://doi.org/10.1016/j.biomaterials.2013.10.072

Yu, Y.P., Wang, Q., Liu, Y.C. and Xie, Y.J.B. (2014), "Molecular basis for the targeted binding of RGD-containing peptide to integrin αVβ3", **35**(5), 1667-1675. https://doi.org/10.1016/j.biomaterials.2013.10.072

Zitzmann, S., Ehemann, V. and Schwab, M. (2002), "Arginine-glycine-aspartic acid (RGD)-peptide binds to both tumor and tumor-endothelial cells in vivo", *Cancer Res.*, **62**(18), 5139-5143.

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