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Original article

Determination of 3D structure and molecular interaction for mir-135b and its silencer as Triple Negative Breast Cancer (TNBC) biomarkers

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Abstract

Triple Negative Breast Cancer (TNBC) is considered as the most dangerous breast cancer type in women and the most difficult to be medicated due to its unresponsive nature towards drug treatments. However, as transcriptomics studies are becoming more advanced, the research on non-coding (nc)RNAs like miRNA and siRNA is being considered as a more feasible approach to deal with TNBC. The mir-135b, a newly found miRNA that has a role in the molecular mechanism of TNBC, is studied mainly for its function as a possible biomarker and a drug candidate. The molecular interaction and structures are still unknown and determining them is the objective of this research. This research utilizes the RNAComposer webservice for 3D RNA structure prediction and the AutoDockTool for molecular docking to determine their interaction. The result shows satisfactory illustration of mir-135b and its siRNA structures with feasible bindings between them. It is shown that the bindings are strong enough to maintain silencing of the gene. However, the molecular docking result shows reversible interaction based on the binding energy meaning its biomarker potential requires combination with other special drug delivery systems. It could be inferred that the siRNAs have potential to be developed in the wet laboratory settings.

Keywords: TNBC, miRNA, mir-135b, molecular docking, biomarker

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Introduction

Breast cancer is understood to be the most dangerous and common form of cancer in women ("WHO | Breast cancer: prevention and control", n.d.). Among the many types of breast cancer, Triple Negative Breast Cancer (TNBC) is one of the most dangerous and deadly. TNBC is known to be hard to deal with due to its tendency of being unresponsive towards multiple therapeutic agents (Hudis & Gianni, 2011). However, there might be possibility to find novel diagnostics and medication approaches to the increasingly advancing transcriptomics approach. Several miRNAs, which are short non-coding RNAs that regulate gene expression, have been found to be a potential biomarker in breast cancer, such as miRNA-451 and miRNA-155 (Kovalchuk et al., 2008; Kong et al., 2013). This approach commonly uses the application of small interfering RNAs (siRNAs) to study the molecular mechanism of TNBC which works by blocking the expression of the target gene (Deng et al., 2013).

The mir-135b is amongst one of the newly found mRNA to have influence in basal-like TNBCs and can become a potential biomarker due to its role in the TGF-beta, WNT, and ERBB pathways that correlates with breast cancer pathogenesis (Uva et al., 2018; Jamdade et al., 2015). mir-135b is found to be upregulated in basal-like TNBC and this affects multiple biological processes in the body because it deregulates its target genes and helps cause the aggressiveness of the cancer. Some of the target genes include ER, AR, HIF1AN, and LATS2 which are known to be deregulated in breast cancer due to their ability in being tumor suppressor genes (Aakula et al., 2015; Hua et al., 2016).

In recent years, researchers have found a promising biological process to treat diseases like cancer, viral infections, and autoimmune disease called RNA Interference (RNAi). RNAi is an RNA-mediated natural gene regulatory process that involves siRNA which are 21-25 nucleotides long double-stranded RNA (dsRNA) with 2 nucleotides 3'-overhangs (Devi, 2006; Nikam & Gore, 2018). RNAi process results in cleavage of the target mRNA which facilitates gene silencing and as such can be used as potential disease biomarkers. Even though efficient and specific gene silencing through siRNA is extremely promising, there are still challenges that need to be overcome, such as stability of expression, off-target silencing, and immune response. The most common problem is maintaining the stability of the siRNA that will be injected into the patient as they are highly susceptible to serum and tissue enzymes such as RNA degrading nuclease (Gavrilov & Saltzman, 2012). To this extent, predicting the stability of mRNA-siRNA interaction is a very important step in designing siRNA drugs/treatments. Therefore, there is a gap that a finer-grained siRNA is necessary to inhibit the gene that play role in the progression of TNBC.

Molecular simulation methods, such as 2D and 3D modeling of possible drug candidates and novel biomarkers have been found to play an important role in creating a blueprint in drug design (Kinjo and Nishikawa, 2005; Arnold et al., 2006). These methods can be applied for transcriptomics-based biomarkers research as well.

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The objective of this research is to predict the 3D structure of mir-135b with its respective siRNA to create the initial blueprint of its drug design process. The second objective of this research is to determine the interaction of mir-135b with its complimentary siRNA to see its effectiveness as a biomarker and a possible cancer drug treatment. It is expected that the siRNA would eventually downregulate the mir-135b expression when it is forwarded to the wet laboratory setting.

Method

Material

Nucleotide sequence for mir-135b and its siRNA had been obtained in previous research utilizing an established pipeline by Parikesit (2018) using the Vienna RNA Package website in here http://rna.tbi.univie.ac.at/. The 3D model pipeline was employed to confirm the graphical results are provided in vivid resolution. The molecular docking pipeline used was a free Graphical User Interface (GUI) software which also provided graphical results of the binding interaction between mir-135b and its siRNA. It could be downloaded in here: https://autodock.scripps.edu/. The utilized software and hardware were the standard Windows 7 based notebook with a broadband internet connection.

3D modelling

The 3D structure prediction of mir-135b and its siRNA were generated using the online webservice tool called RNAComposer which can be found at http://rnacomposer.cs.put.poznan.pl/ (Antczak et al., 2016). The human mir-135b sequence obtained from NCBI (https://www.ncbi.nlm.nih.gov/nuccore/NR_029893.1?report=fasta) and its siRNA sequence obtained from the RNAxs Tool in Vienna RNA Package website at http://rna.tbi.univie.ac.at//RNAxs/RNAxsrYcxOw/NR_029893.1HOMOSAPIENSMICRORNA135BMIR135BMICRORNA_RNA/ (Gruber et al., 2014) were both forwarded separately into RNAComposer to obtain the respective 3D structure. The Protein Data Bank (PDB) files were created for molecular docking analysis.

RNA-RNA interaction & molecular docking

Interaction between both RNA molecules was determined and visualized using the RILogo Web Server found at https://rth.dk/resources/rilogo/ (Menzel et al., 2012). This was done to initially predict how the siRNA molecule would interact with mir-135b before it was visualized with the molecular docking analysis. The AutoDockTools software version 1.5.7 (http://autodock.scripps.edu/) was implemented as the molecular docking analysis tool due to its graphical results availability for ease of visualization (Morris et al., 2009). The mir-135b PDB file was prepared in prior as the PDBQT molecule file format along with the siRNA as the ligand in the same format. Both file formats were prepared following the requirement for docking (polar hydrogen and Kollman charges addition). The grid box used was based on the mir-135b's active site previously predicted by the RNAxs Tool. The conformation results indicating different possible interactions between mir-135b and its siRNA were obtained in the form of a table containing binding energy values predicted by the tool under default parameters.

Data analysis & interpretation

The 3D files were visualized, and their structural conservation was observed. The mean Binding Energy scores from the molecular docking analysis were used to predict the spontaneity and permanence of the RNA molecular reaction.

Results

Human (*Homo sapiens*) mir-135b sequence was used as the base in this research since Human TNBC is the target of the research. Results for the 2D conserved structure of mir-135b and its siRNA are available in the previous study (Valeska & Parikesit, 2020).

3D model visualization

The YASARA Visualization Tool (Krieger & Vriend, 2014) was utilized to visualize the 3D model of mir-135b and its siRNA obtained from RNAComposer **(Fig. 1)**. YASARA was chosen due to its compatibility with most forms of PDB files, including nucleotide 3D structure format. The data restriction to only RNA inputs prevented the de novo models from binding to other proteins.

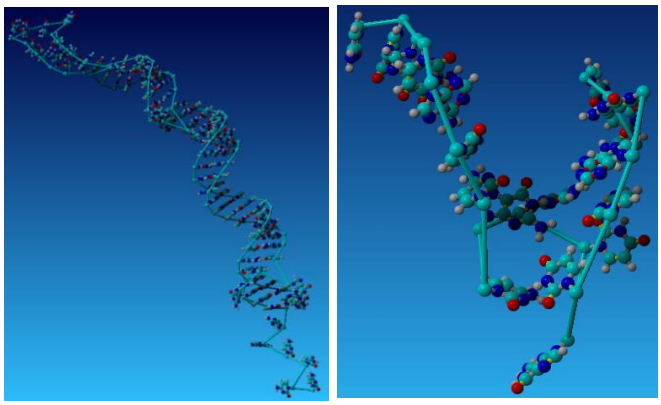

Figure 1. Visualization of 3D models by YASARA. Left: mir-135b, Right: mir-135b's siRNA.

RNA interaction & molecular docking analysis

Results from the RILogo Web Server showed only intramolecular reactions and no intermolecular reactions between both RNA molecules **(Fig. 2)**. Intramolecular reactions show that hydrogen bonds exist in each RNA molecule and is possibly what governs their folding, however, the result was unable to predict the reaction between both molecules. This is possibly due to the incomplete annotation of the web server's database and as such a deeper analysis is required in the form of molecular docking analysis.

The molecular docking analysis used default parameters given by the AutoDockTools itself without any changes and the ligand (siRNA) was limited to using no torsions. This is because AutoDock has a maximum of 32 torsions in 1 ligand, however, the ligand used in this research had a total of 146 torsions due to siRNA in gen-

eral being highly unstable. The docking analysis was run 10 times to make sure all the possible bindings/interactions between the 2 molecules. Results showed a consensus cluster conformation from all 10 runs **[Fig. 3]** meaning the parameters chosen were enough to provide accurate results (Morris & Lim-Wilby, 2008). Assessment of similarity between the cluster conformations was determined by the RMSD threshold below 2Å. RMSD values from all 10 conformations are below the threshold signifying all 10 conformations are similar enough to be grouped together as 1 cluster.

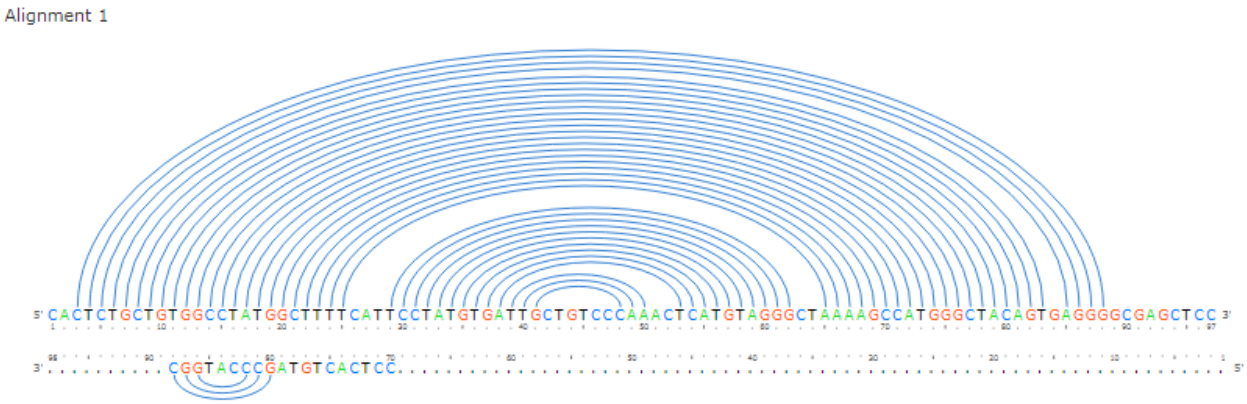

Figure 2. RNA-RNA interaction of mir-135b and its siRNA visualized from RILogo Web Server. Intramolecular reaction is shown in blue-colored arcs.

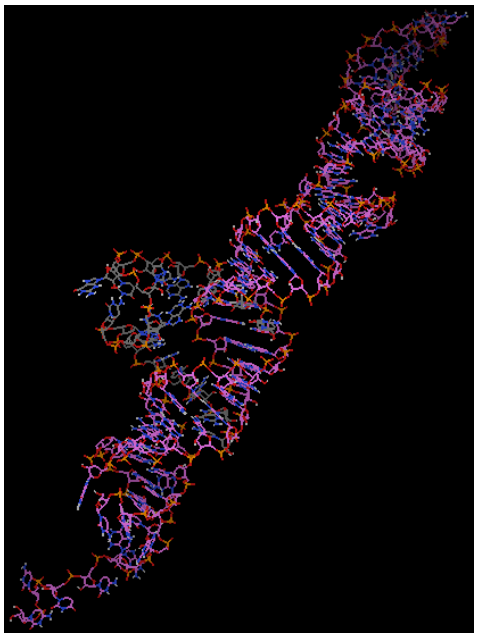

Figure 3. mir-135b and its siRNA best docking conformation viewed from AutoDockTools. Purple-red: mir-135, Blue-red: mir-135b's siRNA.

Discussion

It is already known that mir-135b is upregulated in TNBC patient, and this research is devising pipeline for designing siRNA that could potentially downregulated the micro RNAs. In this regard, the conformations of siRNAs play important role to inhibit the mir-135b. The conformation chosen as the best and closest to the observed crystallography binding was determined using the binding energy since it can predict the biochemical reaction between 2 molecules based on thermodynamics law. Based on that, the lowest binding energy would be the best and the closest result. However, all 10 conformations showed an extremely high positive binding energy while negative binding energy is usually preferred **(Table 1)**. In AutoDock4, high or positive binding energy is interpreted as reversible and non-permanent interaction. In this regard, the negative binding energy or high energy score is catered for a permanent interaction between siRNA and mir-135b.

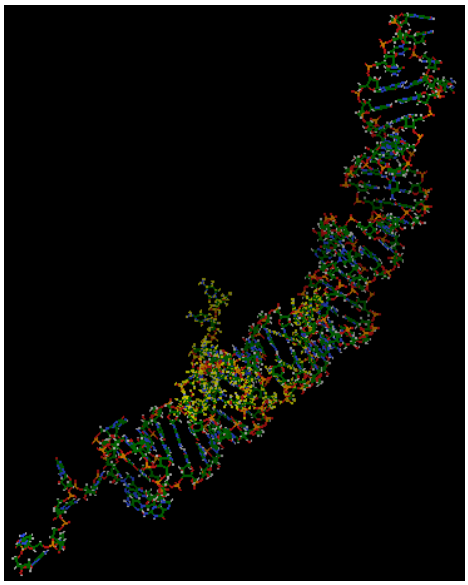

Figure 4. PatchDock result of mir-135b and its siRNA visualized using AutoDockTools. Large molecule: mir-135b, small molecule highlighted yellow: mir-135b's siRNA.

In this case, the extremely positive binding energy values showed that siRNA's interaction with mir-135b is highly reversible, meaning that it is an unstable reaction. However, the full mechanism and biochemical reaction between both RNAs has not been fully deciphered yet, meaning there is a possibility to stabilize the reaction once the proper catalytic or autocatalytic mechanism is found. The simulated interaction between siRNA and mir-135b in here was depicted in reductionist setting that did not count into account some other possibilities of

catalytic mechanism to boost the kinetic of the reactions. This result was further validated using PatchDock, another tool for molecular docking analysis available in the form of a web server which can be found at http://bioinfo3d.cs.tau.ac.il/PatchDock/ (Schneidman-Duhovny et al., 2005). Results from PatchDock when visualized provided a similar result as AutoDock showing the consistency of the analysis done using the set parameter mentioned previously **(Fig. 4)**. In this regard, the high binding values correspond to the negative free energy that made possible for permanent binding between mir-135b and the designed siRNA. It expected that the simulated binding will incite the downregulation of mir-135b that would eventually mediate the regression of TNBC. More studies in this area is needed, especially in the field of drug delivery that could ensure the on-targeting of the siRNA to the gene or the micro RNA.

The 3D structure of mir-135b and its siRNA can be elucidated using molecular modeling software to produce results in a fine-grain resolution. It was also found to be a possible TNBC biomarker and a drug candidate through molecular docking methods. However, further analysis may be done to stabilize the reaction between both molecules through their catalytic mechanism.

Table 1. Summary of AutoDockTools docking results ranked based on binding energy values.

Rank	Run	Binding Energy	Cluster RMSD
1	10	+306702.95	0.00
2	3	+306884.85	0.02
3	7	+306988.47	0.01
4	9	+307012.93	0.04
5	1	+307015.48	0.02
6	6	+307087.01	0.02
7	4	+307176.13	0.02
8	2	+307263.33	0.02
9	5	+307474.88	0.01
10	8	+307506.87	0.05

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List of abbreviations

siRNA: small interfering RNA
miRNA: micro RNA

References

Aakula, A., Leivonen, S.-K., Hintsanen, P., Aittokallio, T., Ceder, Y., Børresen-Dale, A.-L., Perälä, M., Östling, P., & Kallioniemi, O. (2015). MicroRNA-135b regulates ERα, AR and HIF1AN and affects breast and prostate cancer cell growth. *Molecular Oncology*, *9*(7), 1287–1300. doi: 10.1016/j.molonc.2015.03.001

Antczak, M., Popenda, M., Zok, T., Sarzynska, J., Ratajczak, T., Tomczyk, K., Adamiak, R.W., & Szachniuk, M. (2016). New functionality of RNAComposer: an application to shape the axis of miR160 precursor structure. *Acta Biochimica Polonica*, *63*(4), 737–744. doi: 10.18388/abp.2016_1329

Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2005). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*, *22*(2), 195–201. doi: 10.1093/bioinformatics/bti770

Deng, Z. J., Morton, S. W., Ben-Akiva, E., Dreaden, E. C., Shopsowitz, K. E., & Hammond, P. T. (2013). Layer-by-Layer Nanoparticles for Systemic Codelivery of an Anticancer Drug and siRNA for Potential Triple-Negative Breast Cancer Treatment. ACS Nano, 7(11), 9571–9584. doi: 10.1021/nn4047925

Devi, G. R. (2006). siRNA-based approaches in cancer therapy. *Cancer Gene Therapy*, *13*(9), 819–829. doi: 10.1038/sj.cgt.7700931

Gavrilov, K., & Saltzman, W. M. (2012). Therapeutic siRNA: Principles, Challenges, and Strategies. *Yale Journal of Biology and Medicine (YJBM)*, *85*(2), 187–200.

Gruber, A. R., Bernhart, S. H., & Lorenz, R. (2014). The ViennaRNA Web Services. RNA *Bioinformatics*, 307–326. doi: 10.1007/978-1-4939-2291-8_19

Hua, K., Jin, J., Zhao, J., Song, J., Song, H., Li, D., Maskey, N., Zhao, B., Wu, C., Xu, H., & Fang, L. (2016). miR-135b, upregulated in breast cancer, promotes cell growth and disrupts the cell cycle by regulating LATS2. *International Journal of Oncology*, *48*(5), 1997–2006. doi: 10.3892/ijo.2016.3405

Hudis, C. A., & Gianni, L. (2011). Triple-Negative Breast Cancer: An Unmet Medical Need. *The Oncologist*, *16*(Supplement 1), 1–11. doi: 10.1634/theoncologist.2011-s1-01

Jamdade, V. S., Sethi, N., Mundhe, N. A., Kumar, P., Lahkar, M., & Sinha, N. (2015). Therapeutic targets of triple-negative breast cancer: a review. British Journal of *Pharmacology*, *172*(17), 4228–4237. doi:10.1111/bph.13211

Kinjo, A. R., & Nishikawa, K. (2005). Recoverable one-dimensional encoding of three-dimensional protein structures. *Bioinformatics*, *21*(10), 2167–2170. doi: 10.1093/bioinformatics/bti330

Kong, W., He, L., Richards, E. J., Challa, S., Xu, C.-X., Permuth-Wey, J., Lancaster, J. M., Coppola, D., Sellers, T. A., Djeu, J. Y., & Cheng, J. Q. (2013). Upregulation of miRNA-155 promotes tumour angiogenesis by targeting VHL and is associated with poor prognosis and triple-negative breast cancer. *Oncogene*, *33*(6), 679–689. doi: 10.1038/onc.2012.636

Kovalchuk, O., Filkowski, J., Meservy, J., Ilnytskyy, Y., Tryndyak, V. P., Chekhun, V. F., & Pogribny, I. P. (2008). Involvement of microRNA-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. *Molecular Cancer Therapeutics*, *7*(7), 2152–2159. doi: 10.1158/1535-7163.mct-08-0021

Krieger, E., & Vriend, G. (2014). YASARA View – molecular graphics for all devices – from smartphones to workstations. *Bioinformatics*, *30*(20), 2981–2982. doi: 10.1093/bioinformatics/btu426

Menzel, P., Seemann, S. E., & Gorodkin, J. (2012). RILogo: visualizing RNA–RNA interactions. *Bioinformatics*, *28*(19), 2523–2526. doi: 10.1093/bioinformatics/bts461

Morris, G. M., & Lim-Wilby, M. (2008). Molecular Docking. *Methods in Molecular Biology*, *443*, 365–382. doi: 10.1007/978-1-59745-177-2_19

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. *J. Computational Chemistry*, *30*(16), 2785–2791. doi: 10.1002/jcc.21256

Nikam, R. R., & Gore, K. R. (2018). Journey of siRNA: Clinical Developments and Targeted Delivery. *Nucleic Acid Therapeutics*, *28*(4), 209–224. doi: 10.1089/nat.2017.0715

Parikesit, A. A. (2018). The Construction of Two and Three Dimensional Molecular Models for the miR-31 and Its Silencer as the Triple Negative Breast Cancer Biomarkers. *OnLine Journal of Biological Sciences*, *18*(4), 424–431. doi: 10.3844/ojbsci.2018.424.431

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., & Wolfson, H. J. (2005). PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Research*, *33*, W363–W367. doi: 10.1093/nar/gki481

Uva, P., Cossu-Rocca, P., Loi, F., Pira, G., Murgia, L., Orrù, S., Floris, M., Muroni, M. R., Sanges, F., Carru, C., Angius, A., & De Miglio, M. R. (2018). miRNA-135b Contributes to Triple Negative Breast Cancer Molecular Heterogeneity: Different Expression Profile in Basal-like Versus non-Basal-like Phenotypes. *International Journal of Medical Sciences*, *15*(6), 536–548. doi: 10.7150/ijms.23402

Valeska, M. D., & Parikesit, A. A. (2020). Structural Bioinformatics Approach for the Molecular Models of mir-135b and Its Silencer as Triple Negative Breast Cancer (TNBC) Biomarkers. In H. S. Watanabe (Ed.), *Horizons in Cancer Research*, *Volume 77*. Nova Science Publishers.

WHO | Breast cancer: prevention and control. (n.d.). Retrieved from https://www.who.int/cancer/detection/breastcancer/en/