Generating Two-Dimensional Repertoire of siRNA Linc-ROR and siRNA mRNA ARF6 from the lincRNA-RoR/miR-145/ARF6 expression Pathway that involved in the progression of Triple Negative Breast Cancer

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Abstract. The research for finding the cure for breast cancer is currently entering the interesting phase of the transcriptomics based method. With the application of Next Generation Sequencing (NGS), molecular information on breast cancer could be gathered. Thus, both in silico and wet lab research has determined that the role of lincRNA-RoR/miR-145/ARF6 expression Pathway could not be ignored as one of the cardinal starting points for Triple-Negative Breast Cancer (TNBC). As the most hazardous type of breast cancer, TNBC should be treated with the most advanced approach that available in the scientific community. Bioinformatics approach has found the possible siRNA-based drug candidates for TNBC. It was found that siRNA that interfere with lincRNA-RoR and mRNA ARF6 could be a feasible opportunity as the drug candidate for TNBC. However, this claim should be validated with more thorough thermodynamics and kinetics computational approach as the comprehensive way to comprehend their molecular repertoire. In this respect, the claim was validated using various tools such as the RNAfold server to determine the 2D structure, Barriers server to comprehend the RNA folding kinetics, RNAeval server to validate the siRNA-target interaction. It was found that the thermodynamics and kinetics repertoire of the siRNA are indeed rational and feasible. In this end, our computation approach has proven that our designed siRNA could interact with lincRNA-RoR/miR-145/ARF6 expression Pathway.

1. Introduction

As autocatalytic biomolecules, RNA has deemed as the possible origin of ‘last universal common ancestor’ (LUCA) [1]. Thus, slowly but sure, RNA-based flow of genetic information has supplemented the accepted protein-based dogma central theory [2,3]. However, this working pipeline still upheld by protein biomolecules such as DICER and DGCR [4]. Still, up to now, the role of RNA-based information flow is getting more important as the mechanism of non-coding (nc)RNA become unfolded [2,5]. Recent studies have shown that RNA or transcriptomics based diagnostics and drug candidates have been developed extensively for cancer [6–8]. Specifically, the role of silencing (si)RNA as short interference transcriptome, has been considered heavily as drug candidates [9,10]. The relative thermodynamics and kinetics versatility of siRNA are important contributing factors for the formulation integrity of the biomolecules [11]. Ground-breaking computational methods have been devised for designing the siRNA molecules, and it is greatly assisted with RNA Vienna Package [12,13]. This versatile package has been applied for transcriptomics annotations of virus, human, plants, and animals [14–16]. Those studies encourage us to apply the package into the breast cancer research.

As one of the most dangerous malady for woman, breast cancer is currently only curable via conventional medication approaches, namely radiotherapy and chemotherapy [17]. In Indonesia, breast cancer is also considered one of the deadliest diseases for woman [18]. However, current
progress in breast cancer medication also points to the biomolecular approach, especially towards the transcriptomics methods [19–21]. Thus, in this respects, interest on how to obtained fine-grained repertoire on the exact biomolecular mechanism of the RNA molecules also grows [11,22]. Based on its biomolecular mechanism, breast cancer has four types, namely: luminal A, luminal B, Her-2, and triple negative breast cancer (TNBC). Concerning TNBC, which is considered the most dangerous type, it is regulated with lincRNA-RoR/miR-145/ARF6 expression pathway [23,24]. Computational measures to design siRNA based on lincRNA-RoR/miR-145/ARF6 pathway has been devised, hence without good resolution for its biomolecular trajectory [25]. Hence, Vienna RNA package already provided tools to measure the trajectory in efficient manner such as RNA fold for structure elucidation, Barriers server to comprehend the RNA folding kinetics, RNAeval server to validate the siRNA-target interaction [10,26]. In this end, the objective of this research is to annotate the trajectory of siRNA drug candidate for TNBC.

2. Material and Methods
The data was taken from existing publication, in the form of siRNA design [25]. It is elucidated as FASTA sequences and ‘dot bracket’ structural elucidation. Thus the online version of Vienna RNA Package in http://rna.tbi.univie.ac.at was accessed to utilized biomolecular repertoire annotation tools [27]. There are two siRNA designs that currently annotated, namely siRNA for lincRNA-RoR and ARF6. The sequences and structural elucidations were forwarded to Barriers server, RNA fold and RNAeval server which are parts of online Vienna RNA Package [11,14,28]. The tools would be utilized with their respective default parameters. The final step would be analyzing the thermodynamics and kinetics data in order to determine the exact trajectories of the biomolecules.

3. Results and Discussion
The repertoire data was shown with RNAfold and Barrier server program as part of Vienna RNA Package. RNAfold successfully provided 2D visualization of siRNA molecules, as shown in figure 1. The base pair probability in the both siRNA molecules of Figure 1 means the homology between query siRNA with the template on the database. The color scale from 0 to 1 refers to the probability of structural formation. As seen in Figure 1, due to the very high probability of structural formation, the round-shape structure is possibly occurred. The probability of structural formation indicator is comparable with the entropy of the biomolecules themselves.

Figure 1: RNAfold 2D structure prediction of a) siRNA lincROR b) siRNA ARF6
Thus, the siRNA lincROR free energy of thermodynamics ensemble is -0.06 kcal/mol, while for siRNA ARF6 is 0.00 kcal/mol. Although the reaction is near to inert thermodynamics threshold, it is indeed spontaneous as shown in the folding repertoire. Moreover, the animation of the structural transition could be annotated and displayed as well. It is shown on Figure 2. In the left side of the figure, the base pairing occurred within respecting complementary bases. The pairing process would be projected to the structural formation on the right side. The top most figure is showing the thermodynamics repertoire of the structural transition. The structure that pointed in the peak shows the most feasible structure.

Figure 2: Barrier server trajectory of siRNA lincROR. The panel is animated in the computer.

For siRNA lincROR, RNAsubopt predicted 29 structures in an energy range of 40 kcal/mol above the minimum free energy. Then, for siRNA ARF6, RNAsubopt predicted 7 structures in an energy range of 8 kcal/mol above the minimum free energy. Whole predicted structures were spontaneous. The siRNA ARF6 was able to bind with its respective target RNA as shown from the total amount of total free energy binding of -19.80 kcal/mol. Meanwhile siRNA lincROR was able to bind with its respective target RNA as shown from the total amount of total free energy binding of -35.59 kcal/mol. In this end, the bindings were shown as spontaneous.

Although siRNA-based lead compound is very promising for drug development, there is certain concern that should be addressed. As RNA based compound, siRNA is prone to degradation by RNAse enzyme in our cells [29]. This concern should be addressed with more sophistication in drug delivery methods[30]. Some possible approaches are cyclization, using sterically stable liposome formation, and utilization of polymeric nanoparticles [31–33]. Although those methods were mainly developed for peptide and synthetic lead compounds, it is also feasible to incorporate them in siRNA lead compounds development. However, although in vitro and in vivo approach for siRNA drug delivery has provided useful results, the clinical trials are still need many improvements [9,34,35]. For further enhancements, scientist is trying to integrate nanotechnology with current drug design methods [36]. In this end, it would take some years from now until siRNA-based drugs become common in the market.
4. Conclusion

The siRNA structure elucidation for silencers of Linc-ROR and ARF 6 have proven to be feasible in thermodynamics and kinetics terms. Vienna RNA package has proven to be able to annotate the 2D structure of siRNA. The future venue of this research should be devised on the drug delivery methods of the lead compound, in order to reach the target cells safely.

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