J Genet Resour 2024; 10(1): 40-45 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2024.26594.1382*

J|G|R

Bioinformatic Prediction of Novel microRNAs Encoded in Krüppel-like Factor 4 Gene

Mina Zahiri[1], Maryam Hassanlou[1*], Amir-Reza Javanmard [2*] and Nooshin Bijari[3]

[1] Farzanegan Campus, Semnan University, Semnan, Iran
[2] Molecular Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[3]Faculty of Biological Sciences, Semnan University, Semnan, Iran

ARTICLE INFO

Article history:
Received 07 August 2023
Accepted 21 September 2023
Available online 07 October 2023

Keywords:
Bioinformatics software
Non-coding RNAs
Pluripotency
Stem cell

Corresponding authors:
✉ M. Hassanlou
m.hassanlou@semnan.ac.ir
✉ A. Javanmard
amirjavan1372@gmail.com

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

MicroRNAs are small non-coding RNAs that can regulate gene expression that affects various cellular processes. Krüppel-like factor 4 (KLF4) is a transcription factor that has different regulatory functions, and it plays a role in various cellular processes. This study aims to identify novel microRNAs in KLF4 gene using bioinformatics tools. This study significantly contributes to our understanding of the complex function of KLF4 and reveals additional layers of regulatory complexity that affect gene function and cellular dynamics. Advanced bioinformatics methods, including SSCprofiler website, were used to predict stem-loop structures in KLF4 gene, and MatureBayes website was used to predict the mature sequence of microRNAs, which indicate potential miRNA candidates. Using the RNAfold website, the stem-loop structure of microRNAs was determined. The UCSC database assessed the conservation status of these miRNAs and their precursors. From the results of bioinformatics analysis of KLF4 gene, three microRNAs were predicted. Websites and bioinformatics tools were able to predict the sequence of possible microRNAs along with their mature sequence and then depict their stem-loop structure. The analysis of the obtained sequences showed that they are highly conserved, which indicates their importance in the genome. The results obtained in this study show the power and functionality of bioinformatics tools. While the bioinformatic results increase our understanding of the function of the KLF4 gene, experimental studies are needed to confirm these results, which can give us more information about the function of this gene in the future.

Please cite this paper as: Zahiri, M., Hassanlou, M., Javanmard, A., & Bijari, N. (2024). Bioinformatic prediction of novel microRNAs encoded in Krüppel-like factor 4 gene. *Journal of Genetic Resources*, *10*(1), 40-45. doi: 10.22080/jgr.2024.26594.1382

Introduction

MicroRNAs, which are 18 to 25 nucleotide short non-coding RNAs, bind to mRNA and regulate the post-transcriptional expression of the gene (Simonson and Das, 2015). Mature miRNAs bind to the complementary 3'-UTR of their particular target mRNAs after joining the RISC complex (Moreno-Moya *et al*., 2014) Nucleotides 2 to 7 from the 5' end of the miRNA, also referred to as the seed sequence, pair with the complementary sequence of the mRNA to enable miRNAs to bind to their target mRNAs specifically (Quévillon Huberdeau and Simard, 2019). Although a variety of factors may influence miRNA-mRNA binding, it is generally accepted that the miRNA's seed sequence controls both the degree to which miRNA binds to mRNA and the impact it has on mRNA expression (Mullany *et al*., 2016). A single miRNA can target hundreds of mRNAs and

affect the expression of many genes that are often involved in a functionally interacting pathway (Lu and Rothenberg, 2018). It has been predicted that 50% of all protein-coding genes in mammals are controlled by miRNAs (Krol *et al*., 2010).

The human body's undifferentiated cells are called stem cells (Zakrzewski *et al*., 2019). The two primary properties of stem cells are pluripotency, or the capacity to differentiate into various cell types, and self-renewal (Kolios and Moodley, 2012). Cells of all three germ layers can be differentiated from pluripotent stem cells (PSCs), which can proliferate endlessly (Yamanaka., 2020). Transiently aberrant overexpression of Oct4, Sox2, Nanog, KLF4, and MYC transcription factors can program somatic cells to become induced pluripotent stem cells (Yang *et al*., 2020). According to certain theories, KLF4 plays a role in the early stages of reprogramming by promoting the expression of epithelial genes. This reprogramming is thought to be the consequence of pluripotent factors jointly activating a mesenchymal-epithelial transition (MET) program (Hayashi *et al*., 2014).

Krüppel-like factor 4 (KLF4) is a zinc finger-containing transcription factor involved in the regulation of cell growth, proliferation, differentiation, invasion, autophagy, and embryogenesis (Blum *et al*., 2021). KLF4's primary function in a cell is to promote survival by inhibiting apoptosis (Ghaleb and Yang, 2017). Nevertheless, it was subsequently discovered that KLF4 may switch from an anti-apoptotic to a pro-apoptotic function in specific circumstances (Ghaleb and Yang, 2017). KLF4 protein is involved in DNA binding, gene activation, and gene repression through its three unique functional domains (Park *et al*., 2016). KLF4 controls cell division by binding to particular DNA sequences, such as GC and CACCC boxes (Luo *et al*., 2022). For instance, KLF4 deficiency in the mouse esophagus can promote epithelial cell proliferation (Li *et al*., 2021). This gene is conserved in all vertebrate species, including humans and zebrafish (Ghaleb and Yang, 2017). In 2009, Judson and colleagues reported that the introduction of specific microRNAs, namely miRNA-291-3p, miRNA-294, miRNA-295, and miRNA-302d, significantly enhanced the generation of mouse induced pluripotent stem cells (iPSCs) when combined with the transcription factors Oct4, Sox2, and Klf4. Notably, miRNA-291-3p, miRNA-294, and miRNA-295 belong to the miRNA-290 cluster, which is known for its elevated expression in embryonic stem cells (ESCs) and its crucial role in various biological processes related to pluripotency and early embryonic development (Zeng *et al*., 2018). KLF4 dysfunction has been linked to several human illnesses, most notably cancer (Wang *et al*., 2020).

In this study, novel microRNAs transcribed from the KLF4 gene were predicted using various bioinformatics tools and websites.

Materials and Methods

Prediction of miRNA in KLF4

The investigation into putative microRNA (miRNA) structures within the KLF4 gene was conducted using the SSCprofiler platform (http://mirna.imbb.forth.gr/SSCprofiler.html).

This computational tool employs a multifaceted analysis, incorporating features such as sequence, structural characteristics, and sequence conservation. The discernment of potential hairpin structures was contingent upon specific criteria: a restriction on the number of protrusions (<16), loops (<32), an average of loops and protrusions (<37), and a preservation threshold of over 25% of nucleotides. Diverging from tRNAs and rRNAs, pre-miRNAs were distinguished by a lower folding free energy compared to random sequences. Ensuring the veracity of the predicted hairpin structures necessitated minimum free energy, as defined by RNAfold, below -44.25 kcal/mol. The scoring threshold of 3 was applied, with higher scores indicative of a heightened likelihood of accurate predictions (Dokanehiifard *et al*., 2017).

Prediction of miRNA secondary structure

The RNAfold platform was employed to predict the secondary structure of miRNA sequences (http://rna.tbi.univie.ac.at/). This computational tool calculates the Minimum Free Energy (MFE) structure and represents it color-coded based on base pair probabilities, ranging from 0 to 1. A higher probability is denoted by red, while lower

probabilities are visualized in blue (Dokanehiifard *et al*., 2017).

Conservation analysis of miRNA

To assess the conservation of predicted miRNA, the BLAT section of the UCSC site (http://genome.ucsc.edu/) was utilized. The conservation assessment was instrumental in elucidating the potential functional significance of identified miRNAs (Dokanehiifard *et al*., 2017).

Prediction of mature miRNA sequences

The MatureBayes tool (http://mirna.imbb.forth.gr/MatureBayes.html) was employed to determine mature miRNA sequences. This tool provides two options for computing the most probable start positions of mature miRNAs within each miRNA precursor. The integration of these computational methodologies establishes a comprehensive framework for unraveling miRNA presence, structural characteristics, and potential regulatory roles within the KLF4 gene. The insights garnered herein lay the foundation for subsequent experimental inquiries into the intricate regulatory networks governed by miRNAs in this genomic locus (Dokanehiifard *et al*., 2017).

Results

Prediction of miRNA in KLF4

The comprehensive bioinformatic analysis utilizing the SSCprofiler site successfully identified three miRNAs within the KLF4 gene, namely miR-1-KLF4, miR-2-KLF4, and miR-3-KLF4 (Table 1). Subsequently, the MatureBayes website was employed to predict the mature sequences of these identified miRNAs, providing additional insights into their molecular characteristics (Table 1).

miRNAs stem-loop structure and their conservations

Upon subjecting the pre-miRNA sequences to thorough examination via the UCSC website, the analysis unveiled the existence of three miRNAs within the KLF4 gene, demonstrating notable saddle-like conservation across 17 distinct species. Scores range from 0 to 1, with higher scores corresponding to higher levels of conservation. The predictive prowess of the RNAfold website was then harnessed to elucidate the stem-loop structures of these miRNAs, providing visual representations of their structural motifs (Fig. 1). This dual-faceted approach not only highlights the structural features of the miRNAs but also underscores their evolutionary conservation.

Discussion

The method used in this study has already been used for the bioinformatic identification of miRNAs such ashsa-miR-6165 (Parsi *et al*., 2012) PIK3CA-miR1 (Saleh *et al*., 2016) TrkC-miR1 (Dokanehiifard *et al*., 2015) etc., which after prediction, using experimental methods, their existence has been confirmed. With the development of bioinformatics, the path of predicting and discovering biological molecules such as microRNAs has become easier (Gomes et al., 2013). In this bioinformatics study, three microRNAs that are probably transcribed from the KLF4 gene were predicted by the mentioned websites.

Table 1. Predicted miRNAs, Pre-miRNA sequences from SSCprofiler and mature miRNA sequences from Maturebayes located in the KLF4 gene.

miRNA	Pre-miRNA (5′→ 3′)	Mature miRNA (5′→ 3′)
miR-1	ACCGGCGCCTACTTTTCTGCCCAGGAGCCTTTTGCTTGA TGGATGGTATCTGTGTTTGAATGTGCAATTTTGTTAGGC TTTTAAAAGGCAGGAGATTCCCCGCG	miR-1-5p: GAGCCUUUUGCUUGAUGGAUGG miR-1-3p: UGUGCAAUUUUGUUAGGCUUUU
miR-2	TTTTGGCTTCGTTTCTTCTCTTCGTTGACTTTGGGGTTCA GGTGCCCCAGCTGCTTCGGGCTGCCGAGGACCTTCTGG GCCCCCACATTAATGAGGTAGGTGAG	miR-2-5p: GGUUCAGGUGCCCCAGCUGCUU miR-2-3p: CGAGGACCUUCUGGGCCCCCAC
miR-3	CTCCCTTTCTTCTCTCCGCCCCCCCCGAGGCTCCCTTCC ATCGTTGCTATGGCAGCTAAATCAACAAACTCGGCGCA CGTGggggcggggagggggaaggaggg	miR-3-5p: UCUCUCCGCCCCCCCCGAGGCU miR-3-3p: CGGCGCACGUGGGGGCGGGGGA

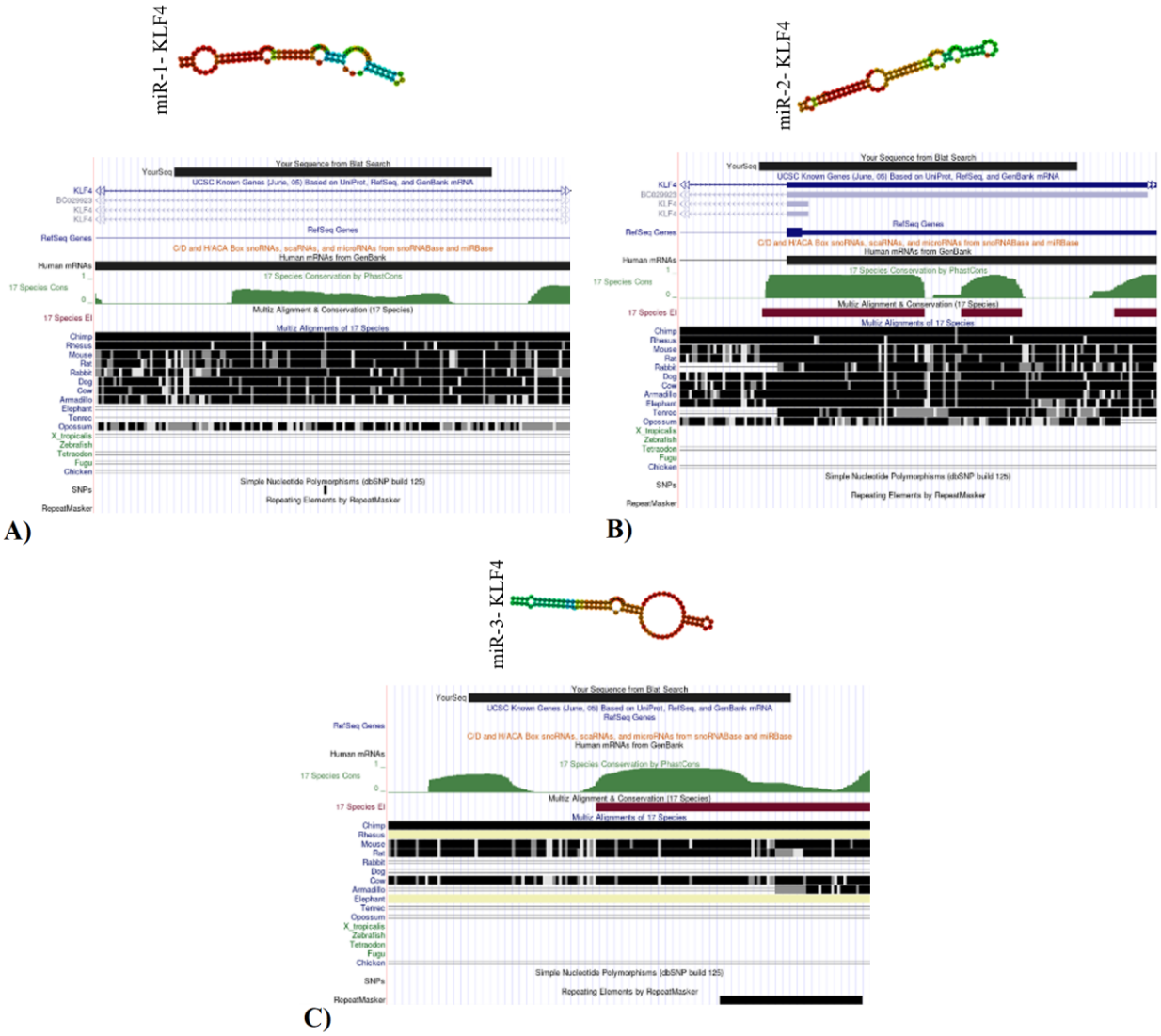

Fig. 1. Prediction of stem-loop structure of 3 KLF4 miRNAs and its conservations.

Predicting novel microRNAs with innovative methods can help to better understand the regulatory systems within the cells. Parts of the KLF4 gene, in which the predicted microRNAs are transcribed, showed a high degree of conservation, which indicates the great importance of that part of the sequence. The prediction of the possible structure of microRNAs showed the stem-loop structure, which is one of the characteristic features of microRNAs. To date, no microRNA that is transcribed from the KLF4 gene has been predicted or discovered. As a result, the findings of our study can help expand our knowledge of the role of microRNAs. In this study, powerful bioinformatics tools were used, which led to the prediction of microRNAs. The results of our bioinformatic studies need experimental confirmation, which, in the case of future studies, can confirm our findings and complete the bioinformatic evaluation by conducting practical tests.

Conclusion

In this study, three possible microRNAs that are transcribed from the KLF4 gene were predicted using bioinformatics tools. The results of bioinformatics websites were able to predict microRNA stem-loop structure and their mature structure. While experimental studies are needed to confirm these microRNAs, the results of this study can be the beginning of a way to better

understand the function of this gene in different cells and even types of diseases.

Author Contributions

M.Z., M.H.; conception, design, analysis and interpretation of the data A.-R.J., N.B., M.H.; approval of the final version. All authors have read and agreed to the published version of the manuscript.

Disclosure Statement

The authors declare that there is no conflict of interest regarding this article.

References

Blum, A., Mostow, K., Jackett, K., Kelty, E., Dakpa, T., Ryan, C., & Hagos, E. (2021). KLF4 regulates metabolic homeostasis in response to stress. *Cells*, 10(4), 830. https://doi.org/10.3390/cells10040830

Dokanehiifard, S., Soltani, B. M., Parsi, S., Hosseini, F., Javan, M., & Mowla, S. J. (2015). Experimental verification of a conserved intronic microRNA located in the human TrkC gene with a cell type-dependent apoptotic function. *Cellular and Molecular Life Sciences*, 72, 2613-2625. https://doi.org/10.1007/s00018-015-1868-4

Dokanehiifard, S., Yasari, A., Najafi, H., Jafarzadeh, M., Nikkhah, M., Mowla, S. J., & Soltani, B. M. (2017). A novel microRNA located in the TrkC gene regulates the Wnt signaling pathway and is differentially expressed in colorectal cancer specimens. *Journal of Biological Chemistry*, 292(18), 7566-7577. https://doi.org/10.1074/jbc.M116.760710

Ghaleb, A. M., & Yang, V. W. (2017). Krüppel-like factor 4 (KLF4): what we currently know. *Gene*, 611, 27-37. https://doi.org/10.1016/j.gene.2017.02.025

Hayashi, K., Sasamura, H., Nakamura, M., Azegami, T., Oguchi, H., Sakamaki, Y., & Itoh, H. (2014). KLF4-dependent epigenetic remodeling modulates podocyte phenotypes and attenuates proteinuria. *The Journal of Clinical Investigation*, 124(6), 2523-2537. https://doi.org/10.1172/jci69557

Kolios, G., & Moodley, Y. (2012). Introduction to stem cells and regenerative medicine. *Respiration*, 85(1), 3-10. https://doi.org/10.1159/000345615

Krol, J., Loedige, I., & Filipowicz, W. (2010). The widespread regulation of microRNA biogenesis, function and decay. *Nature Reviews Genetics*, 11(9), 597-610. https://doi.org/10.1038/nrg2843

Li, J. C., Chen, Q. H., Jian, R., Zhou, J. R., Xu, Y., Lu, F., ... & Zhang, H. (2021). The partial role of KLF4 and KLF5 in gastrointestinal tumors. *Gastroenterology Research and Practice*, 2021. https://doi.org/10.1155/2021/2425356

Lu, T. X., & Rothenberg, M. E. (2018). MicroRNA. *Journal of Allergy and Clinical Immunology*, 141(4), 1202-1207. https://doi.org/10.1016/j.jaci.2017.08.034

Luo, X., Zhang, Y., Meng, Y., Ji, M., & Wang, Y. (2022). Prognostic significance of KLF4 in solid tumours: an updated meta-analysis. *BMC Cancer*, 22(1), 181. https://doi.org/10.1186/s12885-022-09198-9

Moreno-Moya, J. M., Vilella, F., & Simón, C. (2014). MicroRNA: key gene expression regulators. *Fertility and Sterility*, 101(6), 1516-1523. https://doi.org/10.1016/j.fertnstert.2013.10.042

Mullany, L. E., Herrick, J. S., Wolff, R. K., & Slattery, M. L. (2016). MicroRNA seed region length impact on target messenger RNA expression and survival in colorectal cancer. *PloS One*, 11(4), e0154177. https://doi.org/10.1371/journal.pone.0154177

Park, C. S., Shen, Y., Lewis, A., & Lacorazza, H. D. (2016). Role of the reprogramming factor KLF4 in blood formation. *Journal of Leucocyte Biology*, 99(5), 673-685. https://doi.org/10.1189/jlb.1RU1215-539R

Parsi, S., Soltani, B. M., Hosseini, E., Tousi, S. E., & Mowla, S. J. (2012). Experimental verification of a predicted intronic microRNA in human NGFR gene with a potential pro-apoptotic function. *PloS One*, 7(4), e35561. https://doi.org/10.1371/journal.pone.0035561

Quévillon Huberdeau, M., & Simard, M. J. (2019). A guide to microRNA-mediated gene silencing. *The FEBS Journal*, 286(4), 642-652. https://doi.org/10.1111/febs.14666

Saleh, A. J., Soltani, B. M., Dokanehiifard, S., Medlej, A., Tavalaei, M., & Mowla, S. J. (2016). Experimental verification of a predicted novel microRNA located in human

PIK3CA gene with a potential oncogenic function in colorectal cancer. *Tumor Biology*, 37, 14089-14101. https://doi.org/10.1007/s13277-016-5264-y

Simonson, B., & Das, S. (2015). MicroRNA therapeutics: the next magic bullet? *Mini Reviews in Medicinal Chemistry*, 15(6), 467-474. https://doi.org/10.2174/1389557515666150324123208

Wang, X., Xia, S., Li, H., Wang, X., Li, C., Chao, Y., ... & Han, C. (2020). The deubiquitinase USP10 regulates KLF4 stability and suppresses lung tumorigenesis. *Cell Death and Differentiation*, 27(6), 1747-1764. https://doi.org/10.1038/s41418-019-0458-7

Yamanaka, S. (2020). Pluripotent stem cell-based cell therapy-promise and challenges. *Cell Stem Cell*, 27(4), 523-531. https://doi.org/10.1016/j.stem.2020.09.014

Yang, L., Shi, P., Zhao, G., Xu, J., Peng, W., Zhang, J., ... & Cui, H. (2020). Targeting cancer stem cell pathways for cancer therapy. *Signal Transduction and Targeted Therapy*, 5(1), 8. https://doi.org/10.1038/s41392-020-0110-5

Zakrzewski, W., Dobrzyński, M., Szymonowicz, M., & Rybak, Z. (2019). Stem cells: past, present, and future. *Stem Cell Research and Therapy*, 10(1), 1-22. https://doi.org/10.1186/s13287-019-1165-5

Zeng, Z. L., Lin, X. L., Tan, L. L., Liu, Y. M., Qu, K., & Wang, Z. (2018). MicroRNAs: important regulators of induced pluripotent stem cell generation and differentiation. *Stem Cell Reviews and Reports*, 14, 71-81. https://doi.org/10.1007/s12015-017-9785-6