Computation analysis to identify hsa-microRNA-1225-5p from the head and neck squamous cell carcinoma (HNSCC) human genome sequence.

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Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is the eighth most frequent cancer in people worldwide. Finding HNSCC biomarkers is crucial for enhancing patient care by enabling earlier patient diagnosis and treatment. Interestingly, microRNAs (miRNAs or miRs) are studied for their potential role as biomarkers and therapeutic targets in various diseases, including OSCC.

Methodology: In this study, we have used bioinformatic approaches to identify hsa-miR-1225-5p for HNSCC using NCBI database, miRbase and target scan. Finally, RNA fold was used to create the secondary structure of hsa-miR-1225-5p.

Results and discussion: Careful evaluation of the secondary structure result showed that hsa-miR-1225-5p has a minimum free energy of – 48.20 kcal. The correlation between hsa-miR-1225-5p and HNSCC genome sequence was identified.

Conclusion: These computational approaches have concluded that hsa-miR-1225-5p can be used as a diagnosis, prognosis and effective therapeutic target for treating HNSCC. Thus, further research could enlighten the role of hsa-miR-1225-5p in HNSCC.

Keywords: Head and neck squamous cell carcinoma; microRNAs; biomarkers; therapeutic target; hsa-miR-1225-5p

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the eighth most frequent cancer in people worldwide. In addition to using tobacco and drinking alcohol, genetic and epigenetic changes are crucial in the development and occurrence of HNSCC. In addition, it has been reported that 80% to 90% of patients with HNSCC have a history of heavy drinking and smoking, and some have also had papillomavirus infections [1]. Chemotherapy, radiation, and surgery are common

HNSCC treatment options. Due to the disease's heterogeneity, the clinical symptoms of HNSCC vary in the early stages of the tumour's development [2]. Therefore, finding HNSCC biomarkers is crucial for enhancing patient care by enabling earlier patient diagnosis and treatment.

Small noncoding RNAs called microRNAs (miRNAs) interfere with gene expression to control various biological processes, including cell cycle, proliferation, differentiation, and apoptosis [3]. The large class of small single-stranded non-coding endogenous RNAs known as miRNAs interacts with the 3′UTR of target mRNA to regulate genes post-transcriptionally. They range in size from 18 to 25 nucleotides. When compared to normal cells, some miRNAs are either up- or down-regulated in tumour cells [4]. MiRNAs are important players in the development and carcinogenesis of cancers. Several miRNAs are studied for their potential role in the disease progression of HNSCC. Yet there is a lack of knowledge on the disease mechanism which has made the diagnosis and treatment difficult [5-8]. Hence there is a need to identify novel miRNA involved in the carcinogenesis of HNSCC which could act as biomarker and therapeutic target for HNSCC.

In this study, we examined the miRNAs that are eccentrically expressed in HNSCC. The miRBase also serves as a gateway for information from outside sources about miRNA genes and sequence, linking to other sources like those that list predicted and experimentally verified microRNA targets. Thus the miRNA identified could be a potential biomarker and therapeutic target for HNSCC.

Materials and methods

In this study, we used the bioinformatics approach to identify the miRNAs in the HNSCC genome sequence, where the data was collected from publicly accessible databases.

Retrieval of HNSCC sequences and miRNAs

Human genome sequence data was obtained through the National Center for Biotechnology Information (NCBI) web portal for International Nucleotide Sequence Database Consortium. The search term keyword "Head and Neck squamous cell carcinoma genome sequence in Homo sapiens" was used to extract the HNSCC genome sequence using this free search engine. After removing the low-quality and redundant sequences, a local nucleotide database was formed for HNSCC specific genome sequences. Human pre-miRNA (38,589 as of 2022) and mature miRNA (48,885 as of 2022) were retrieved from the miRBase. miRNAs reported from human were used as a reference sequence (http://www.mirbase.org/). The above HNSCC nucleotide database was searched for their homolog among the miRNAs dataset [9].

Identification of precursor-miRNAs

The mature miRNAs were utilised as a starting point for searching the HNSCC nucleotide database for homologs. Reference miRNA sequences were utilised as a query for homology searches against the newly developed local HNSCC-specific nucleotide sequence database at an e-value threshold of 0.01, with all other parameters set to default, using the Basic Local Alignment Search Tool (BLAST) 2.2.26+. All candidate sequences were stored in Fast Alignment (FASTA) format, and the reference precursor and mature miRNA sequences were matched against the singleton dataset using ClustalW. (multiple sequence alignment tool). BLAST against the NCBI protein database with the default value was used to validate sequences with no more than three mismatches for their non-protein encoding phenomena. Then the aligned portion was expressed as a candidate pre-miRNA sequence [9].

Validation of candidate pre-miRNA and their target

The secondary structure was obtained using RNAfold which provided the mature miRNA sequence expressed in the HNSCC genome sequence. The following criteria must be confirmed 1) RNA structure must have an appropriate stem loop hairpin structure 2) mature miRNA must be in one side of the hairpin structure 3) miRNA should have less than 7 mismatches with the opposite miRNA in the other arm 4) secondary structure must have a higher negative energy and A+U content (40-70%) [9]. Target scan was used in target prediction that helped in identification of potential targets. Table 1 represents the criteria for confirmation of RNA structure.

S.no	Criteria
1	RNA structure must have an appropriate stem loop hairpin structure
2	Mature miRNA must be in one side of the hairpin structure
3	miRNA should have less than 7 mismatches with the opposite miRNA in the other arm
4	Secondary structure must have a higher negative energy and A+U content (40-70%)

Results

Identification of pre-miRNA and its secondary structure

miRNAs are involved in the gene expression that regulates the disease progression and regression. Thus, identification of miRNA responsible for HNSCC could aid in early diagnosis and treatment of disease. The miRNA identification was performed through computational approach. The HNSCC human genome sequences were retrieved from the NCBI database and the precursor miRNAs were retrieved from the miRbase. After the collection of sequences and careful evaluation of the secondary structure, one miRNA that is hsa-miR-1225-5p was

identified in the hypertension genome sequences. The mature sequence of hsa-miR-1225-5p was found using RNA fold with the minimum free energy – 48.20 kcal.

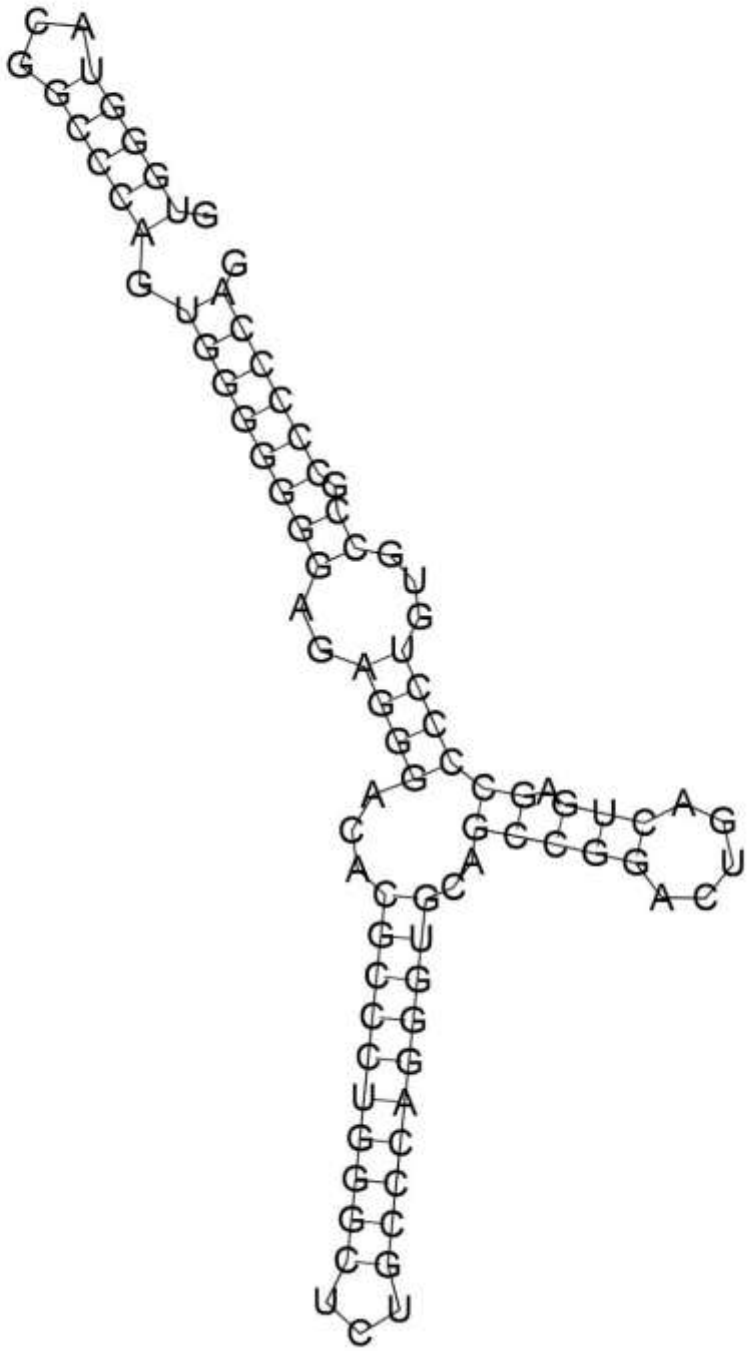

Figure 1

Source miRNA	Source organism	Pre-miRNA length	Minimum Free Energy	Mature Sequence	Match Extent	Strand	A+U%
miR-1225-5p	*Homo sapiens*	90	- 48.20 kcal	GUGGGUACGGCCCAGUGGGGGG	22/22	5p	25.5%

Table 2

Figure 1 represents the secondary structure of hsa-miR-1225-5p. Table 2 displays the pre-miRNA length, minimum free energy, mature sequence, match extent, and A+U% content of hsa-miR-1225-5p.

Identification of targets

Target scan was performed to identify the targets for the specific miRNA. Based on target scan analysis, we identified other important transcripts that are targeted by hsa-miR-1225-5p are zinc finger protein 37A, RNA binding motif protein 38, phospholipid transfer protein, interferon-gamma, etc.

S.no	Target Protein	Representative transcript	Molecular function	Biological process
1	zinc finger protein 37A	ENST00000685332	DNA binding	Transcription
2	phospholipid transfer protein	ENST00000372431	ceramide binding	Lipid transport
3	interferon, gamma	ENST00000229135	Adaptive immune response	Cellular response

5	transmembrane protein 185B	ENST00000426077	DNA binding	Immune response, cytokinesis

Table 3

Table 3 represents the target genes of hsa-miR-1225-5p with their molecular function and biological process

Discussion

The hidden physiological position of HNSCC makes early diagnosis difficult and results in the majority of patients having advanced-stage diagnoses. Local recurrence, cervical node metastases, and low therapeutic response rates to radiation and chemotherapy are the main causes of the high mortality rate in HNSCC. Furthermore, the five-year survival rate is low, which highlights the importance of early diagnosis and treatment. An urgent need exists to boost survival in HNSCC without raising toxicity [10]. On the other hand, miRNAs are short non-coding RNAs that are suitable candidates as biomarkers and therapeutic targets for HNSCC [2,4]. The identified miR-1225-5p is found to be involved in the disease progression of various diseases.

For instance, in a study by Zhang et al. (2020), low miR-1225-5p expression was discovered to be associated with a poor prognosis in patients with osteosarcoma. Sox9 was discovered as a target gene to clarify the mechanism by which miR-1225-5p prevents the growth of osteosarcoma. Exogenous Sox9 expression also had effects on miR-1225-5p's anticancer effects on osteosarcoma cells. By targeting Sox9, the findings collectively imply that miR-1225-5p acts as a tumour suppressor in osteosarcoma and identifies new osteosarcoma therapeutic targets [11].

One of the studies on glioblastoma identified that because of their negative correlation, FNDC3B and miR-1225-5p might be potential target genes for each other. These findings laid the groundwork for the molecular diagnosis and treatment of glioblastoma by demonstrating that the miR-1225-5p/FNDC3B axis inhibits the malignant phenotype of glioblastoma cells [12].

In 2020, Li and his colleagues studied the role of miR-1225-5p in non-small cell lung carcinoma (NSCLC). The findings showed that when compared to healthy control tissues, NSCLC tissues had significantly lower expression levels of miR-1225-5p. Furthermore, it was found that miR-1225-5p may function as an independent prognostic factor in NSCLC. In contrast to miR-1225-5p overexpression, which had the opposite effects in these cells, miR-1225-5p inhibition increased cell proliferation, migration, and invasion in NSCLC cell lines. The results of the study concluded that miR-1225-5p might be a potential therapeutic target for NSCLC because its expression levels in NSCLC were found to be downregulated, which may indicate a poor prognosis for patients [13]. Thus miR-1225-5p is an important miRNA involved in various diseases.

Conclusion

Thus, the present study identified novel miR-1225-5p from the HNSCC human genome sequence. Further studies on the target genes and signalling pathways regulated by them could elucidate the role of miR-1225-5p in HNSCC. Hence, there is a need for further analysis to validate the miR-1225-5p as a potential biomarker and therapeutic target for HNSCC.

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