Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 March; 6 (3s): 958 -962

In-silico identification of microRNA-1224-5p from the Oral squamous cell carcinoma (OSCC) human genome sequence

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common histotype of head and neck cancer, accounting for 90% of all diagnosed cases. The goal of modern cancer research is to develop less invasive techniques that can provide a more complete picture of the cancer profile and make it simple to track the disease's development and therapeutic response. 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-1224-5p for OSCC using NCBI database, miRbase and target scan. Finally, RNA fold was used to create the secondary structure of hsa-miR-1224-5p.
Results and discussion: Careful evaluation of the secondary structure result showed that hsa-miR-1224-5p has a minimum free energy of – 44.10 kcal. The correlation between hsa-miR-1224-5p and OSCC genome sequence was identified.
Conclusion: These computational approaches have concluded that hsa-miR-1224-5p can be used as a diagnosis, prognosis and effective therapeutic target for treating OSCC. Thus, further research could enlighten the role of hsa-miR-1224-5p in OSCC.

Keywords: Oral squamous cell carcinoma; microRNAs; biomarkers; therapeutic target; hsa-miR-1224-5p.

Introduction:

Oral squamous cell carcinoma (OSCC) is the most common histotype of head and neck cancer, accounting for 90% of all diagnosed cases. Despite advances in cancer prevention, diagnosis, and management, OSCC remains a serious public health problem, with 300,000 new cases diagnosed each year and 145,000 deaths. There is still much to learn about the aetiology of OSCC(1) . It might be multifactorial, meaning that a number of genetic abnormalities and environmental risk factors, such as smoking and drinking, human papillomavirus (HPV) infection, and chronic mechanical trauma, could all be contributing factors. OSCC can develop from normal epithelium with genetically altered keratinocytes or from conditions of the mouth that have the potential to develop into cancer, such as oral leukoplakia, erythroplakia, lichen planus, smoking hyperkeratosis, and submucous fibrosis(2).
The goal of modern cancer research is to develop less invasive techniques that can provide a more complete picture of the cancer profile and make it simple to track the disease's development and therapeutic response (3). Interestingly, microRNAs (miRNAs or miRs) are studied for their potential role as biomarkers and therapeutic targets in various diseases,including OSCC. The large family of single-stranded non-coding RNA known as miRNAs controls post-transcriptional gene expression by binding 19–25 nucleotides. MiRNAs are essential for the control of the cell cycle, differentiation, apoptosis, and migration. Any change in the expression of miRNAs can either suppress tumour growth or act as carcinogens because some miRNAs are upregulated, and some are downregulated during the development of cancer (4,5).
In the present study, we employ a bioinformatics approach to identify a novel miRNA involved in the disease progression of OSCC. The miRNA sequence matching with the OSCC human genome sequence will be identified, and its secondary structure and targets will be analysed.

Materials and methods:

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

Retrieval of OSCC 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 “Oral squamous cell carcinoma genome sequence in Homo sapiens" was used to extract the OSCC genome sequence using this free search engine. After removing the low-quality and redundant sequences, a local nucleotide database was formed for OSCC 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 humans were used as a reference sequence (http://www.mirbase.org/). The above OSCC nucleotide database was searched for their homology among the miRNAs dataset (6).

Identification of precursor-miRNAs

The mature miRNAs were utilised as a starting point for searching the OSCC nucleotide database for homologs. Reference miRNA sequences were utilised as a query for homology searches against the newly developed local OSCC-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(6).

Validation of candidate pre-miRNA and their target

The secondary structure was obtained using RNAfold which provided the mature miRNA sequence expressed in the OSCC 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%) [6]. Target scan was used in target prediction that helped in identification of potential targets. Table 1 represents the criteria for confirmation of RNA structure.

Results:

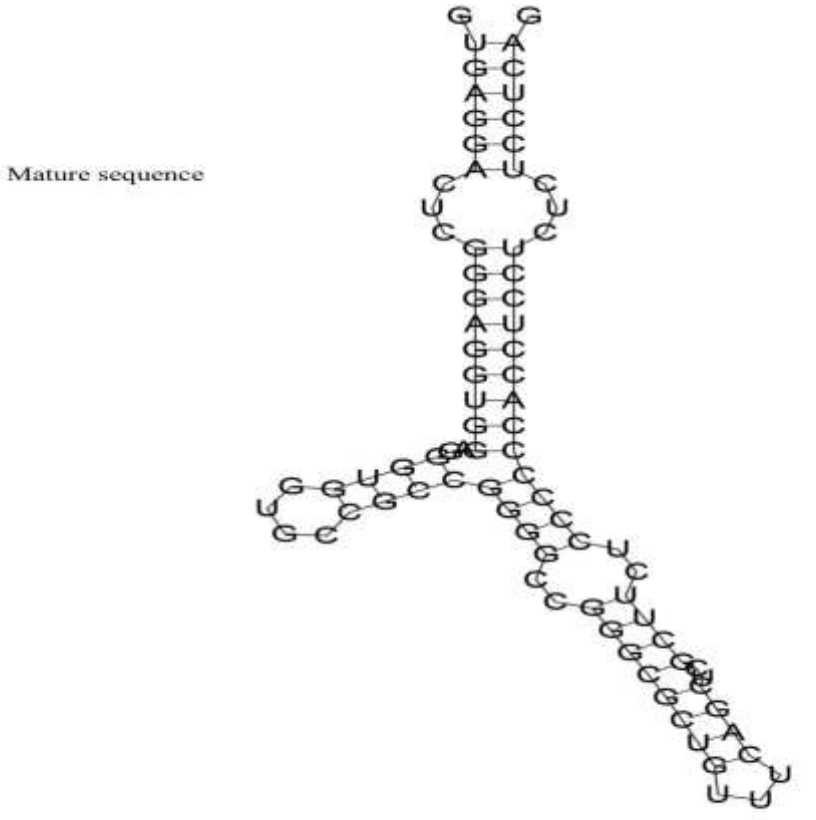

Figure 1 represents the secondary structure of hsa-miR-1224-5p

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%)

Table 2 displays the pre-miRNA length, minimum free energy, mature sequence, match extent, and A+U% content of hsa-miR-1224-5p

Source miRNA	Source organism	Pre-miRNA length	Minimum Free Energy	Mature Sequence	Match Extent	Strand	A+U%
miR-1224-5p	*Homo sapiens*	85	- 44.10 kcal	GUGAGGACUCGG GAGGUGG	19/19	5p	29.4%

Table 3 represents the target genes of hsa-miR-1224-5p with their molecular function and biological process

S.no	Target Protein	Representative transcript	Molecular function	Biological process
1	transforming growth factor, beta receptor II	ENST00000295754	ATP binding	Activation of protein kinase
2	tubulin, beta 2A class IIa	ENST00000333628	GTP binding	Mitotic cell cycle
3	adenosine A1 receptor	ENST00000337894	G-protein coupled receptor binding	Apoptotic signalling pathway
4	zinc finger protein 629	ENST00000262525	Endonuclease, Helicase, Hydrolase, Multifunctional enzyme, Nuclease	DNA damage, DNA repair
5	transmembrane protein 185B	ENST00000426077	DNA binding	Immune response, cytokinesis

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 OSCC could aid in early diagnosis and treatment of disease. The miRNA identification was performed through a computational approach. The OSCC 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-1224-5p was identified in the hypertension genome sequences. The mature sequence of hsa-miR-1224-5pwas found using RNA fold with the minimum free energy – 44.10 kcal.

Figure 1 represents the secondary structure of hsa-miR-1224-5p. Table 2 displays the pre-miRNA length, minimum free energy, mature sequence, match extent, and A+U% content of hsa-miR-1224-5p.

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-1224-5pare transmembrane protein 221, G protein signalling modulator 3, adenosine A1 receptor, pantothenate kinase 1 etc. Table 3 represents the target genes of hsa-miR-1224-5p with their molecular function and biological process.

Discussion:

The most common head and neck cancer is OSCC, which has a poor prognosis due to frequent cervical lymph node metastasis. The goal of the current study was to locate potential markers, specifically microRNAs (miRNAs) and the target genes they bind to, that are highly significant in the aetiology of OSCC (7). The identified novel miR-1224-5p is one of the miRNAs having a matching sequence with the OSCC human genome sequence. Interestingly, miR-1224-5p is identified to be having an important role in the disease progression of glioblastoma (8), acute lung injury (9), colorectal cancer (10)and so on. But there are no studies on the role of miR-1224-5p in OSCC. This is the first study to report miR-1224-5p in OSCC.

Fascinatingly, in a study by Jiang et al. (2021), the expression and relationship of circular RNA (Circ)-RNF121, FOXM1, and miR-1224-5p in colorectal cancer (CRC) tissues or cells were analysed. MiR-1224-5p inhibitors attenuated the effects of Circ-RNF121 silencing, which suppressed cell proliferation, migration, invasion, and glycolysis but increased cell apoptosis in CRC. Additionally, miR-1224-5p and miR-1224-5p bound to FOXM1 were absorbed by circ-RNF121(11). Thus the circ-RNF121/ miR-1224-5p/ FOXM1 axis was identified to have important roles in the disease progression of CRC.

Similarly, another study by Li et al. (2020) in CRC demonstrated how SP1 expression and metastasis are negatively correlated with miR-1224-5p downregulation in CRC in both human patients and xenografted mouse models. Gain- and loss-of-function assays show that miR-1224-5p directly targets SP1 to inhibit CRC cell migration, invasion, and epithelial-mesenchymal transition (EMT) both in vitro and in vivo. Additionally, SP1 encourages the phosphorylation of p65, which advances EMT in CRC cells. According to clinical analysis, miR-1224-5p and SP1 expression are remarkably linked to advanced clinical traits and a poor prognosis in CRC patients. Additional research supports the hypothesis that miR-1224-5p is depleted in CRC due to hypoxia. MiR-1224-5p prevents the development of hypoxia during the epithelial-mesenchymal transition and metastasis of CRC cells (12). Thus, the study concluded that miR-1224-5p is a potential biomarker and therapeutic target for CRC.

Accumulating evidence suggests that miR-1224-5p is a potential tumour-suppressive miRNA involved in the disease progression of various cancers. Thus, further studies on the role of miR-1224-5p in OSCC could help in the understanding of molecular mechanisms and signalling pathways involved in OSCC.

Conclusion:

In conclusion, miR-1224-5p is identified as a novel miRNA whose sequence matches with the OSCC human genome sequence. Thus further in vitro and in vivo analysis could validate miR-1224-5p as a potential biomarker and therapeutic target for OSCC. OSCC is highly prevalent, and the 5-year survival rate of OSCC is quite low. Hence there is an urgent need for studies on the identification of novel biomarkers and therapeutic targets for the diagnosis, prognosis and treatment of OSCC.

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