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ORIGINAL ARTICLE **OPEN ACCESS**

shRNA Inhibits the Abnormal Production of RAD51L1 Proteins in Nasopharyngeal Carcinoma through RNA Interference

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ABSTRACT
Nasopharyngeal carcinoma could be a threat emerging from the epithelium of the nasopharynx. It is curable if diagnosed and treated early. The actual prevalence of nasopharyngeal cancer in Pakistan is about 18.74% of all newly recorded cases of cancer. The average ages of the male (42 years) and females (35 years) have been reported. The incidence ratio among male and female is 3:1. There are numerous medicines against nasopharyngeal cancer, but this research incorporates the novel insilico method of shRNA against RAD5L1 in nasopharyngeal cancer as tight hairpin that can be utilized to target gene expression by means of RNA interference. However, mRNA sequences were retrieved and tandem repeats were removed. Untranslated regions were removed as UTRs are the part of the mRNA sequence that directly follows the translation termination codon. Each mRNA sequences are begins from start codon AUG which are efficient to identify the target sites.In homology search, it is important to remove the homologous target sites, no similar sequence is identified as similar to target sites when binds to the shRNA can block the expression of the normal gene.
Keywords: *shRNA, Epstein bar virus, Untranslated regions, tandem repeats, RAD51L1.*

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INTRODUCTION

Nasopharyngeal carcinoma (NPC), being an undifferentiated type, is the epithelial malignancy grows from the upper portion of the pharynx, extend to the soft palate to the base of the skull. Increase in growth may produce the nasal obstruction or bleeding and nasal twang. NPC is intermediated with Epstein bar virus (EBV) infection result in the malignancy phenotype, viral, environmental and hereditary [1-3]. The factors included viruses are proteins, small RNA and microRNAs as well as when they interact with pathways as cell proliferation. These interactions result in immune recognition and elimination which is beneficial for the survival of viral proteins [4]. This disease tends to affect a younger group of patients early recognition might reduce the chance of Smorbidity and mortality[5]. It can be diagnosed at early stages needing high clinical and cross-sectional investigations on tumor confirmation and histology [6]. The use of clinical investigations of EBV as surrogate biomarkers for NPCs which can continue to increase with quantitative assessment of circulating EBV.For the population screening and disease surveillance, DNA is mainly used. The role of EBV is investigating the random trail in DNA of patients to treat the escalation and augmentations. The main treatment modality consists primarily of radiation therapy whereas cross-sectional imaging is important in order to achieve an accurate delineation of tumor extent, thereby facilitating both staging and treatment. MRI is currently considered the best modality to assess for NPC [7-8].

For locally advanced and or node-positive patients, chemotherapy is presently the best treatment whereas platinum-based treatment is favored for thin restoration method [9]. Several studies show DNA breaks due to the replication of DNA agents that causes the damage of DNA is treats with RAD51L1 through its involvement in HRR. Heterodimer can be formed by RAD51L1 with RAD51C which ahead linked to another member like RAD51, XRCC2, and XRCC3. RAD51 overexpression causes the slowdown of G1 phase and apoptosis of cell, which leads the damage of DNA [10 - 12]. A slighter, there then again spliced transcript variations have been noticed which encodes particular isoforms [13].

shRNA designing is of potential value in gene therapy of NPC by specifically inhibiting cancer associated target gene. As many studies have shown the activation of a targeted gene in a sequence-specific manner via small RNA molecules like microRNAs or dsRNAs rather than silencing it. In this review, the novel phenomenon of RNA interference is used against RAD51L1 to lower the rate of NPC risks.shRNA are designed against hungston disease by to degrade the expression of mutant huntingtin mRNA [14]. However lentiviral delivered shRNA are used for the treatment of HIV / AIDS as anti-tumor bifunctional siRNA (bi-shRNA) and siRNA as for the treatment of metastatic melanoma [15].
This study focuses to inhibit the production of RAD51LA proteins in NPC through a technique known as RNA interference.

MATERIAL AND METHODS

Sequence retrieval

The mutated mRNA sequences of RAD51L1 were retrieved fromhttps://www.ncbi.nlm.nih.gov.The accession number mRNA sequence of RAD51L1 are NM_001321810.1,NM_001321812.1, NM_001321814.1, NM_001321815.1, NM_001321817.1, NM_001321818.1,NM_001321819.1, NM_001321821.1, NM_002877.5, NM_133509.3, NM_133510.3.

Identification of start codons

The start codons were identified and removed from mutated mRNA sequences from codon finder too. The first codon of mRNA sequence is the start codon, from which translation starts.

Identification of target sites

The targeted sites of RAD51L1 mRNA for shRNA were identified via siRNA Target Finder tool available at https://www.genscript.com.

Identification of untranslated regions (UTR) motifs and repeats

The untranslated regions are parts of mRNA that directly follows the translation termination codon. The mRNA molecules were copied from DNA which was translated into proteins. The 3' UTRs were bind to the binding sites for regulatory proteins and microRNAs. Specific binding in 3'utrs miRNA, in turn, decrease the expression of genes mRNA by inhibiting translation or by the degradation of mRNA [16]. The untranslated regions were retrieved from RegRNA server.

Homology search against mRNA databases

In the NCBI Blast database, the homology search was carried out to identify the related sequences for the target regions for each mRNA molecule. Itis necessary to remove from homologous target sites, due to the fact if a comparable collection is present in any gene as a target site there is the chance of binding shRNA to the normal gene resulting in expression inhibition.

The efficient shRNA design

The shRNA had been designed for every targeted sequence via OligoWalk server. The evaluation was completed for each shRNA.

RESULT

This study focus on the selection of eleven mRNA sequences of RAD51L1 genes which were retrieved from the NCBI database. NM-001321810-1 transcript variant 1 RAD51 paralog B consist of two start codons, NM-001321812-1 transcript variant 2 contains three start codons, NM-001321815-1 transcript variant 5 can start translation from two inframe upstream AUG codon while NM-001321817.1 transcript variant 3 and NM-001321818.1 transcript variant 4 RAD51 Paralog B can initiate translation from two in-frame downstream start codons.

The first codon of mRNA Start codon which are transcribed by ribosomes in prokaryotes AUG codes for Met and in eukaryotes this start codon always codes for methionine. The untranslated 5' regions are always preceded by start codon[17].

Each mRNA sequence begins from start codon AUG which is efficient to identify the target sites. Target sites were identified in each mRNA transcript sequence,each mRNA sequence contains a G+C content ratio as shown in Table 1.

TABLE1: The target sites identified against each mRNA sequence with a G+C content

S. No	Sequences	Start position	G + C %	Energies
1	UAAAAACAAAAGUGGAAGC	1758	54%	-20.6
2	UUUUCAACCAGUCUUUCAGC	522	54%	-21.3
3	UAAAAAUCCCAUAUAGAAGC	1861	45%	-24.2
4	UUUCUUUCUUUGAGAUUGC	754	50%	-25.5

shRNA having G+C content ratio lying between 30-55% are more efficient and have great efficiency to act as functionalshRNA as compared to those havinga greater percentage of G+C content., while designing the shRNA, 5' UTR and 3' UTR would be avoided , although these UTRs while targeting the shRNA efficiently induced gene silencing .

The 3' untranslated region is a part of mRNA that continually follows the translation end codon. Not all the genes of mRNA are converted into proteins, including the 5'UTR, 5' cap, the poly(A) tail, and 3'UTR. The 3' UTR continuously have a region of regulation that post-transcriptionally impacts the expression of genes.The 5'UTR is the portion of mRNA that directs to the initiation codon. This portion involves in the regulation of transcription. Each mRNA transcript with similar untranslated regions was identified with the same position. The UTRs are shown in Table 2.

TABLE 2:Theuntranslated regions(UTRs) identified in each mRNA sequence

Motif names	Positions	Lengths	Sequences
SXL binding site	1476 ~ 1487	12	ttttttttttt
Musashi binding element (MBE)	515 ~ 521	7	atttagt
Musashi binding element (MBE)	598 ~ 602	5	gtagt
Musashi binding element (MBE)	1766 ~ 1770	5	atagt

Along with the identification of target sites for shRNA, it is necessary to remove the tandem repeats from each mRNA. A tandem repeat is basically two or more copies of nucleotides. If four or more tandem repeats are present at the end of the target site, the shRNA will not bind to the target, so that each repeat should remove while designing the shRNA.Repeat sequences were found in each mRNA sequence in Table 3.

TABLE 3: Tandem repeats identified and removed from each mRNA

Indices	Period Size	Copy Number	Consensus Size	Percent Matches	Percent Indels	Score	A	C	G	T	Entropy (0-2)
1351--1415	32	2.0	32	100	0	130	27	23	24	24	2.00

In homology search, it is important to remove the homologous target sites, none of the similar sequences was identified as similar to target sites when binds to the shRNA can block the expression of the normal gene. The designed hairpin shRNA consist of sense strands, followed by loops, anti-sense strands and termination signals as shown in Fig 3.

shRNAs are capable to make two complementary 19 – 22 bp sequence of RNA when join with DNA connect with a short loop of 4 – 11 nucleotides like hairpin found in the real miRNA. Antisense stand is positioned 5' or 3' to the loop which follows the unique structural features of shRNA with 19 bp. The efficient shRNA are those having value lower than IC50. In the mechanism of translation,shRNA sequence is moved to the cytosol where it identifies the endogenous protein, dicer, which further follows the complementary duplexes, and this designed shRNA are bind to the target sites of the mRNA and degrade its expression.

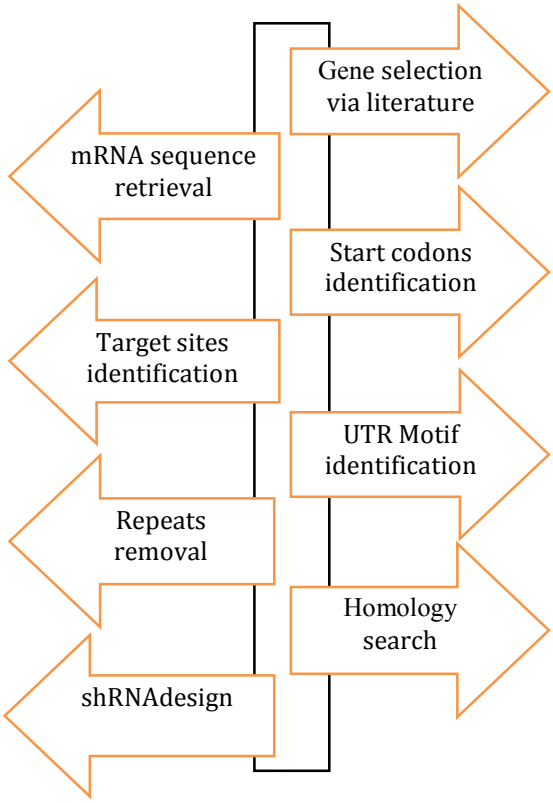

Fig 1: Steps for the designing of shRNA against RAD51L1 of NPCs

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1341 GCATCATAAG

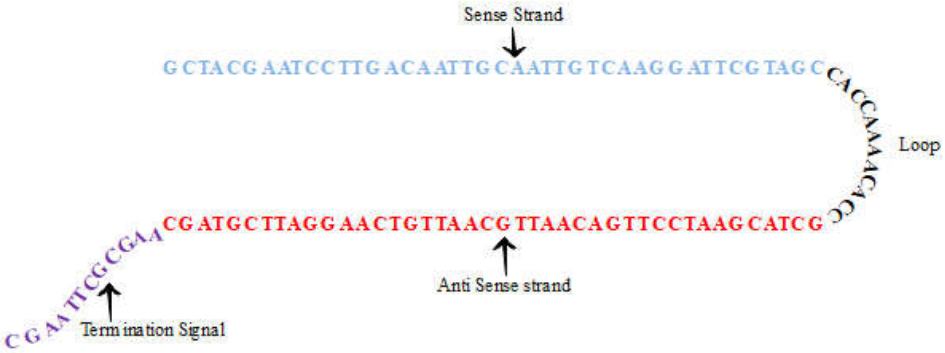

1351 CTGATTTGATACCATGGCACTGACAATGGGCA
1 CTGATTTGATACCATGGCACTGACAATGGGCA


1383 CTGATTTGATACCATGGCACTGACAATGGGCA
1 CTGATTTGATACCATGGCACTGACAATGGGCA


1415 C
1 C

1416 ACAGGGAACA
```

Fig 2: Tandem repeats found between the index 1341 – 1416


Sense Strand
GCTACGAATCCTTGACAATTGCAATTGTCAAGGATTCGTAGC
CACCAAAACAC
Loop
CGATGCTTAGGAACTGTTAACGTTAACAGTTCCTAAGCATCG
Anti Sense strand
CGAATTCGCGAA
Termination Signal


Sense Strand
GGATGTCTTGGGCTTAATTCTAGAATTAAGCCCAAGACATCC
CACCAAAACAC
Loop
CCTACAGAACCCGAATTAAGATCTTAATTCGGGTTCTGTAGG
Anti Sense strand
CGAATTCGCGAA
Termination Signal


Sense Strand
AGGAGAAAGCCAGCTCCATTATAATGGAGCTGGCTTTCTCC
CACCAAAACAC
Loop
TCCTCTTTCGGTCGAGGTAATATTACCTCGACCGAAAGAGG
Anti Sense strand
CGAATTCGCGAA
Termination Signal


Sense Strand
GCCGACATTCGGTCTTCAATATATTGAAGACCGAATGTCGGC
CACCAAAACAC
Loop
GCCGACATTCGGTCTTCAATATATTGAAGACCGAATGTCGGC
Anti Sense strand
CGAATTCGCGAA
Termination Signal

Fig 3: The shRNA hairpin was designed for each mRNA sequence

DISCUSSION

The purpose of this study is to inhibit the production of RAD51L1 proteins in Nasopharyngeal carcinoma through a technique known as shRNA through RNA interference. shRNAs tight hairpin that can be utilized to target gene expression by means of RNA interference. First, we retrieve start codons from mRNAs as start codons are the first codon transcribed by ribosomes. Start codon begins from AUG from where translation starts and through which target site would identify [18]. As adviced by Singh W, while designing the shRNA having more G+C content ratio have great efficiency to act as functional shRNA. As UTRs while targeting the shRNA efficiently induced gene silencing. In homology search, it is important to remove the homologous target sites, no any similar sequence was identified as similar as target sites, and when it binds to the shRNA can block the expression of a normal gene. Researchers can use the shRNA hairpin to splice the expression of the gene [19].

CONCLUSION

In this study, *In silico* designing of shRNA against mutated RAD51L1 gene was done to improve the shRNA possibility to start RNAi. This method takes both time and cost. This study has decided that both computational and in-vitro techniques may be significant for planning the shRNA against RAD51L1 changes. In the future, this computation technique of shRNA will be very helpful to check viability and ampleness.

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CONFLICT OF INTREST

None of the authors have any conflict of interest.

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