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In silico designing of therapeutic small interfering RNA (si RNA) for lynch syndrome silencing

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ABSTRACT

Lynch Syndrome is a hereditary disorder caused by a mutation in a Mismatch Repair gene (*MLH1, MSH2, MSH6, PMS2, and EPCAM-TACSTD1)* which affected persons have a higher than normal chance of increasing *Colorectal Cancer*, *Endometrial Cancer* which also known as *Hereditary Nonpolyposis Colorectal Cancer (HNPCC)*. The mutation of a single gene dramatically increases as well as the chances of contracting cancer increases deletion in mismatch repair gene responsible for lynch syndrome. All genes work in solving mistakes which causes by DNA which is copied in preparation for cell division. The damage in genes prohibit repair of DNA mistakes and as cells segregate, defects stack and uncontrollable cell growth may result in cancer. A total of 60 siRNA were identified *in silico*, in which 6 most potentially siRNA were selected that noted the therapeutic and have high potential of targeted gene silencing in lynch syndrome. These therapeutic siRNA will be useful to knockdown the translation process in targeted gene causing *Lynch syndrome*. The siRNA have great potential to control the cancerous disease.

Keywords: siRNA, Lynch Syndrome, Mismatch repair gene, MLH1, MSH2, MSH6.

INTRODUCTION

Lynch syndrome is also known as hereditary nonpolyposis colorectal cancer (HNPCC) is an inherited disorder that increases the possibility of many types of cancer, mainly cancers of the colon (large intestine), and rectum, which are collectively referred to as colorectal cancer [1,2]. Many people who have lynch syndrome may causes different types of cancers such as stomach, small intestine, liver, gallbladder ducts, upper urinary tract, brain, skin and in women have a high risk of cancer of the ovaries and lining of the uterus (the endometrial). Lynch syndrome may rarely have noncancerous (benign) growths (polyps) in the colon, called colon polyps. In persons with this disorder, colon polyps take place earlier but not in larger figures than they do in the general population [3]. Lynch syndrome runs in families in an autosomal central inheritance pattern. This way that if one close relative carries a gene mutation for this disorder, there's a 50 percent possibility that mutation will be passed on to every kid. The chances of causing lynch syndrome is the same if the mutate gene goes to father or mother to his son or daughter [4]. The genes which inherited in Lynch syndrome are generally causes mistakes in the genetic code, which is made of DNA. Cells produce and segregate, they make number of copy of their DNA and it is not exceptional for some small mistakes to take place. Common cells have mechanisms to distinguish mistakes and repair them. But people who take over one of the abnormal genes linked with Lynch syndrome lack the ability to repair these small mistakes. An addition of these mistakes leads to increasing genetic damage within cells and ultimately can lead to the cells becoming cancerous [5,6]. Colorectal is a cancer from uncontrolled cell growth in the colon in part of large intestine. Genetic analysis shows that essentially colon and rectal tumors are genetically the same cancer [7]. Symptoms of colorectal cancer normally include rectal

bleeding and anemia which are occasionally associated with weight loss and changes in bowel habits. Most colorectal cancer occurs due to lifestyle and increasing age with only a majority of cases connected with fundamental genetic disorders. It usually starts in the facing of the bowel and if left unprocessed, can produce into the muscle layers underneath, and then through the bowel wall. Screening is helpful at decreasing the chance of dying from colorectal cancer and is suggested starting at the age of 50 and continuing until a person is 75 years old. Many genes involve in Lynch syndrome in which variations in the MLH1, MSH2, MSH6, PMS2, or EPCAM gene increase the risk of developing Lynch syndrome [8]. The MLH1, MSH2, MSH6, and PMS2 genes are involved in the repair of mistakes that occur when DNA is copied in preparation for cell division [9]. Mutations in several of these genes prevent the right repair of DNA replication mistakes. As the abnormal cells continue to divide, the accumulated mistakes can lead to uncontrolled cell growth and possibly cancer. Mutations in the EPCAM gene also lead to impaired DNA repair, although the gene is not itself involved in this process. The EPCAM gene lies next to the MSH2 gene on chromosome 2; certain EPCAM gene mutations cause the MSH2 gene to be turned off (inactivated), interrupting DNA repair and leading to accumulated DNA mistakes [10]. Although mutations in these genes predispose individuals to cancer, not all people who carry these mutations develop cancerous tumors [11].

Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is more common in developed countries. Around 60% of cases were diagnosed in the developed world. It is estimated that worldwide, in 2008, 1.23 million new cases of colorectal cancer were clinically diagnosed, and that it killed 608,000 people. Cancers that are confined within the wall of the colon are often curable with surgery while cancer that has spread widely around the body is usually not curable and management then focuses on extending the person's life via siRNA technology and improving quality of life. This discovery led to a surge in interest in harnessing RNAi for biomedical research and drug development.

MATERIALS AND METHODS

Retrieval of gene Sequences- The complete gene sequence of MLH1, MLH3, MSH2, MSH6 and EPCAM of *Lynch Syndrome* Gene were downloaded from NCBI GenBank.

siRNA Designing- The following online programs are used for *in silico* identification of therapeutic siRNA by using the default settings; 1-GenScript siRNA target finder, 2-Whitehead WI siRNA Selection Program, 3-siDESIGN, 4- siRNA wizard3.1, 5- siDirect Version2, 6-siRNA Target Finder. Among these we used GenScript for designing of potential therapeutic siRNA having 21nt length of targeted sequences using the default settings [12].

BLAST against mRNA database- Any off target sequence similarity in other non-targeted organism genomes was evaluated by BLAST against complete GenBank dataset and target site having similarity of more than 16 contiguous base pair with any other organism were eliminated from consideration.

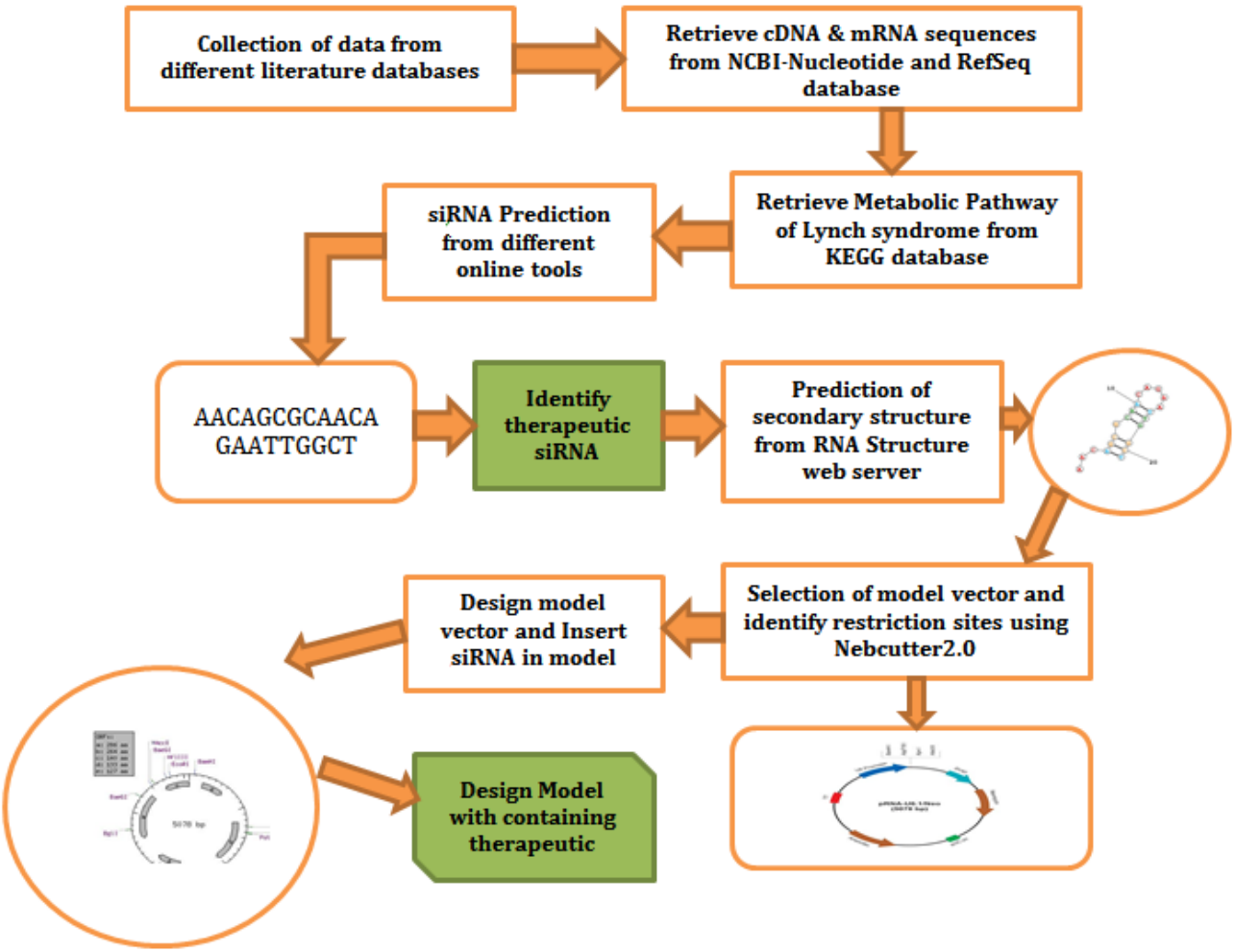
GC Content and siRNA secondary Structure- GC content of selected siRNA was calculated with GenScript and secondary structure were designed by RNA structure web server.

Selection of Model Vector- The suitable expression Vector for the transfer of siRNA was selected from Vector-based siRNAs of siRNA Construct Builder. The marker gene was also selected from the cloning vector pRNA-U6.1/Neo siRNA expression vector. The detailed map showing the position and the features of newly designed vector was obtained.

Table 1. Potential gene involve in Lynch Syndrome selected for designing therapeutic siRNA

Potential Gene	GenBank Accession Number	Important role in Lynch Syndrome
MLH1	U07418.1	This gene was identified as a locus frequently mutated in hereditary nonpolyposis colon cancer.
MLH3	AB039667.1	It is a DNA mismatch repair (MMR) genes that implicated in maintaining genomic integrity during DNA replication and after meiotic recombination
MSH2	U04045.1	This locus is frequently mutated in hereditary nonpolyposis colon cancer (HNPCC). When cloned, it was discovered to be a human homolog of the E. coli mismatch repair gene MutS, consistent with the characteristic alterations in microsatellite sequences
MSH3	J04810.1	It is a part of the post-replicative DNA mismatch repair system. Defects in this gene are a cause of susceptibility to endometrial cancer.
MSH6	U54777.2	The MutS protein helps in the recognition of mismatched nucleotides prior to their repair. The encoded protein heterodimerizes with MSH2 to form a mismatch recognition complex that functions as a bidirectional molecular switch that exchanges ADP and ATP as DNA mismatches are bound and dissociated..
EPCAM	NM_002354.2	This antigen is expressed on most normal epithelial cells and gastrointestinal carcinomas and functions as a homotypic calcium-independent cell adhesion molecule.

Methodology

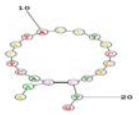
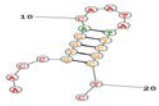


RESULTS AND DISCUSSION

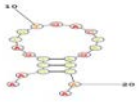
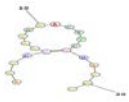
A total of over 60 numbers of siRNA were predicted using GenScript out of which 34 were selected on the basis of low target similarity. The sequences of selected siRNAs are 21 nucleotide long base pair with GC content percents in between 30 to 60. These selected siRNA was modified into siRNA inserts using siRNA construct builder. The siRNA inserts with 76bp in length are generated using siRNA construct builder which includes the antisense region, sense loop, termination signal and restriction enzymes. The siRNA have great potential to control the cancerous disease. **PRNA-U6.1/Neo** siRNA expression vector selected as model vector for insertion siRNA sequences to silence the activity of genes responsible for Lynch syndrome. **pRNA-U6.1/Neo** vector used for transfection in mammalian selected from GenScript siRNA technology containing 5078bp length. The restriction analysis of selected vector and genes of interest is performed using NEBCUTTUR2. Restriction Enzymes used for insertion of siRNA sequences are AccI, AflIII, BaeGI, BamHI, BglI, EcoRI, and PstI to the selected pRNA-U6.1/Neo vector. It carries a neomycin resistance gene that can be used for establishing stable cell line. It uses a U6 promoter for siRNA expression. The detailed map showing the position and the features of newly designed vector was obtained after insertion of siRNA sequences. The siRNA designed in the present study help to control the Lynch Syndrome and cancerous disease by knockdown the process of protein synthesis during the translation. Moreover the all siRNA have the low off target similarity in the published all nucleotide database. These 34 most promising siRNA were selected that address the biosafety concerns and have high potential of targeted gene silencing. These therapeutic siRNA will be useful to knockdown the translation process in targeted gene causing *Lynch syndrome*.

Table 2. List of High potential therapeutic siRNA sequences against Lynch Syndrome Cancers genes, with their sequences features and secondary structures.

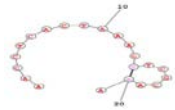
MLH1

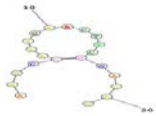
Location of siRNA with in genes.	Min. energy at 37°C	GC Content in %	Target sequence	2D
2065-2085	0.71	47.62	AAGCCTCAGTAAAGAATGCGC	
2428-2448	0.46	38.10	AAGACTTATACTTGCCTTCTG	
462-482	2.86	47.62	AACCATGTGCTGGCAATCAAG	
676-696	0.92	47.62	AACCGTGGACAATATTCGCTC	
1716-1736	0.22	38.10	AACTGTTCTACCAGATACTCA	

MLH3

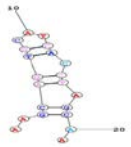
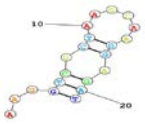
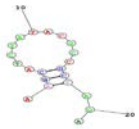
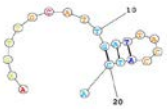
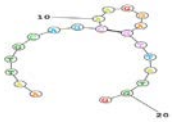
2385-2405	2.17	42.86	AAGAACCGCTTAGAGAACTCT	
2379-2399	2.85	47.62	AAGGACAAGAACCGCTTAGAG	
403-423	2.90	42.86	AAGCTGATGTGACTAGAGCAA	
2465-2485	3.89	47.62	AAGCCACATCCTTGACTCAGA	
3937-3953	2.61	47.62	AAGCCAATGAACTTCGGAGAG	
2810-2830	0.61	42.86	AACATCAGATTCTGCCACACA	

MSH2

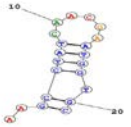
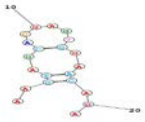
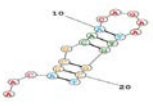
1254-1274	1.35	38.10	AAGAACCGCTTAGAGAACTCT	
1096-1116	0.83	47.62	AAGCCACATCCTTGACTCAGA	

522-542	0.57	52.38	AAGCCAATGAACTTCGGAGAG	
793-813	6.55	42.86	AACATCAGATTCTGCCACACA	

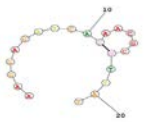
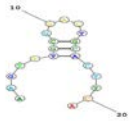
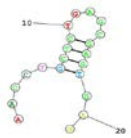
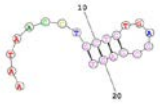
MSH3

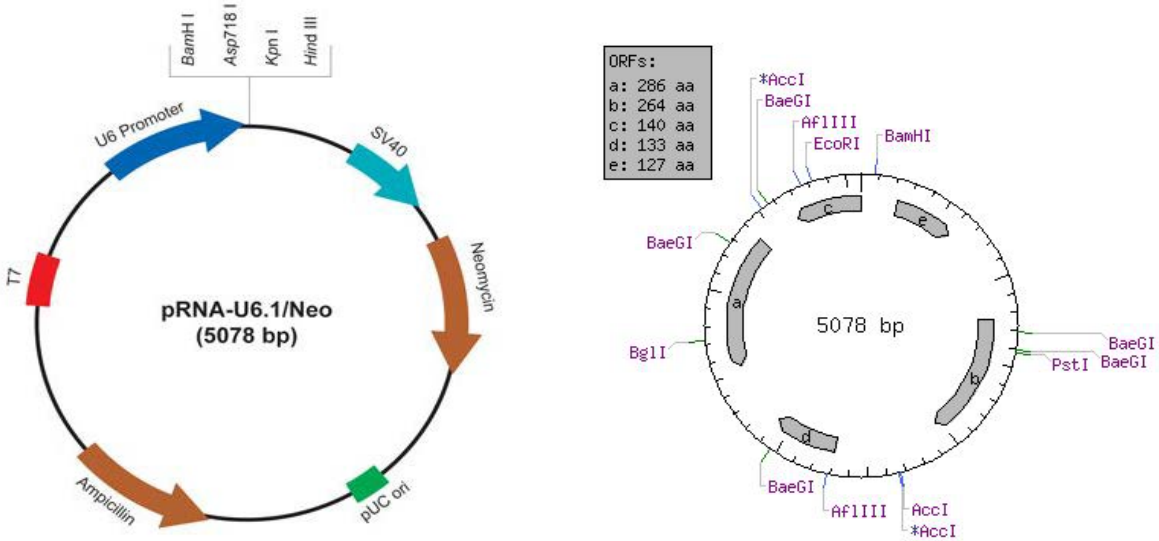
2530-2550	2.79	47.62	AAGCAGTGCATCACCTAGCAA	
1551-1571	6.30	42.86	AAGCCTGTGATTTGCTCTTTG	
2902-2922	3.14	42.86	AAGGACGGAGTACATTTATGG	
2577-2597	4.58	47.62	AAGGTCGCTAAGCAAGGAGAT	
740-760	2.62	47.62	AAGCATCTATACGCCGCTAGA	
2792-2812	0.72	57.14	AAGTTGCATTGATTACCATCA	
3242-3262	1.50	42.86	AATTGCAGCAAGGAGTTATGG	

MSH6

2254-2274	1.12	47.62	AAGCCTATCAACGAATGGTGC	
2458-2478	1.88	52.38	AAGACCTCATGGTTGTGCCTG	
273-293	2.82	52.38	AAGGCGAAGAACCTCAACGGA	
1507-1527	1.93	47.62	AAGTAGCACGAGTGGAACAGA	
2904-2924	1.11	47.62	AACAGCGCAACAGAATTGGCT	

EPCAM

862-882	6.58	42.86	AAGGAGATCACAACGCGTTAT	
840-860	0.22	42.86	AAGTTTGCGGACTGCACTTCA	
759-779	3.73	57.14	AACCTGCTCTGAGCGAGTGAG	
756-776	0.99	47.62	AATAACCTGCTCTGAGCGAGT	
873-893	2.47	47.62	AACGCGTTATCAACTGGATCC	
487-507	3.55	42.86	AATCGTCAATGCCAGTGTACT	
1239-1259	0.94	42.86	AATGGCAAAGTATGAGAAGGC	



A **B**

Figure 1 A. Model vector, B. Newly designed vector

Table 3. Shows features of newly designed Vector

Features of vector	Position
5' LTR	1-75
Polylinker	78 - 101
U6 Promoter	4829 - 77
SV40 Promoter	850 - 1195
Neomycin	1236 - 2030
pUC ori	2744 - 3384
Ampicillin	3532 – 4392
SiRNA Position	840-850 (Depends on Lynch syndrome predicted siRNA length)

CONCLUSION

siRNA gene theraphy is the new tool in biological sciences for the treatment of the various kinds of cancers and diseases. siRNA theraphy is based on the siRNA interference through which double stranded siRNA silence cognate Gene. The present study designed siRNAs for the treatment of lynch syndrome from the selected genes are responsible and designing of suitable vector for the insertion of siRNA sequences which is helpful for the showing expressions of siRNA sequences with the help of various bioinformatics tools.

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