Prioritization of rs187728237 and rs80320514 as miRNA-related Variants of Human *AEG-1* Gene

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ABSTRACT

Background and objectives: 3' untranslated region (3'UTR) single nucleotide polymorphisms (SNPs) represent genetic variations that may potentially affect binding of miRNA to coding genes, potentially leading to complex disorders. We aimed to perform in silico analysis of the potential phenotypic effect of 3'UTR SNPs on human *astrocyte elevated gene-1 (AEG-1)*, a newly identified candidate cancer gene.

Methods: We gathered a list of all 3'UTR SNPs located in the human *AEG-1* gene from the SNP database. Analysis of the potential effects was done using MirSNP and MicroSNiper.

Results: Analysis by the MirSNP estimated that rs187728237 might increase the affinity of two miRNAs and decrease the affinity of 10 other miRNAs to the AEG-1 transcript. Moreover, MicroSNiPer showed that rs80320514 might affect 24 putative miRNA binding sites in the 3'UTR of *AEG-1*.

Conclusion: Based on our findings, it can be concluded that the 3'UTR SNPs located in the human *AEG-1* gene may be within the miRNA targets of the transcript, therefore affecting the stability of putative miRNA-target interactions.

Keywords: AEG-1, miRNA, SNPs, 3' Untranslated Region.

INTRODUCTION

Astrocyte elevated gene-1 (AEG-1) or 3D3/lyric is thought to be associated with several types of cancer, including hepatocellular carcinoma (1-2), breast cancer, malignant gliomas, melanomas (3-4), and neurodegeneration (5). It is also known as human metadherin and a downstream target molecule for *H-Ras* and *c-Myc* (6). Product of the gene induces anchorage-independent growth and tumor cell invasion by increasing the expression of adhesion mediators via NF-κB pathway (6-7). Therefore, it is also considered a direct regulator of angiogenesis (3). In addition, knockdown of *AEG-1* inhibits proliferation of human prostate cancer, neuroblastoma and melanoma cells, and induces apoptosis in prostate cancer and neuroblastoma cells (8). Accordingly, AEG-1 may be a promising target for adjuvant therapy in cancer (9).

AEG-1 mRNA encodes a 582 amino acid protein with a predicted molecular mass of 64 kDa (2, 10), with highly conserved amino acid sequences among vertebrates (11-12). The *AEG-1* gene consists of 12 exons and 11 introns located at chromosome 8q22 (13). MicroRNAs (miRNAs) are non-coding RNAs with 21-25 nucleotides that are key post-transcriptional modulators (14-16). Recent evidence show that even a single miRNA can affect hundreds of genes (17) and become involved in various cellular processes and developmental pathways (18). In addition, they can elevate or reduce expression of protein-coding genes by targeting the 3-untranslated regions (3'UTRs) (19). Seed region residing in mature miRNAs is the crucial binding location for translational regulation (20). The region complementary to the miRNA seed is flexible within the miRNA and regulates mRNA function. Base pairing of the nucleotides in the seed region to complementary sites affects miRNAs length and its interaction with a specific mRNA (21-23).

Single nucleotide polymorphism (SNP) is the most common genetic variation among humans. However, only a minority of SNPs could be considered as markers for underlying susceptibility to different human disorders (24). Among these alterations, SNPs in the seed region of miRNAs may generate/destroy miRNA binding sites, and affect traits and disease pathogenesis (25). Identification of allele-specific miRNA-mRNA interactions could be of great importance for gaining a better understating of the genetics of phenotypic variations. Since experimental analysis is almost impossible for screening all functionally important SNPs, a computational strategy based on biochemical changes and/or sequence information has been proposed for the systematic analysis of SNPs. There is a growing interest in predicting the miRNA-related SNPs for a disease-associated gene that are believed to have no function (26). Considering the continuing increase in frequency of SNPs in miRNA target sites (miRTSs) and lack of enough data on miRNA-related SNPs in the *AEG-1* gene, we aimed to predict SNPs that may potentially affect the miRTSs associated with this gene.

MATERIAL AND METHODS

SNPs in the 3'UTR region of the human *AEG-1* gene and related mRNA accession number (NM178812) were retrieved from the National Center for Biotechnology Information (NCBI) database of SNPs (http://www.ncbi.nlm.-nih.gov/projects/SNP). MicroSNiPer was used to estimate the impact of miRNA binding site SNPs on candidate genes. This online tool provides information about the generation or disruption of putative miRNA binding sites in a gene in presence of alternative SNP alleles. The tool increases flexibility at the input stage. The rs ID interface was also used in the study. MicroSNiPer can generate complementarity between the 5' end of miRNA and its 3'UTR target site, with primary focus on enhancing the specificity of finding the binding sites. In order to filter false positive predictions and capture most human miRTSs, stretches of 7 to 9 nucleotides starting from the 5'-end of the miRNA were selected in the analysis (16). The reliability of miRTS prediction is improved by a requirement for perfect seed pairing, especially when the seed site is conserved in the UTR regions of whole-genome alignments. We also used MirSNP, which utilizes miRanda algorithm for characterizing potential miRNA-related SNPs. All retrieved SNP IDs were searched through MirSNP to obtain prediction results. MirSNP database can also present generation or disruption energy for miRNA hybrid formation in wild-type and mutant alleles, in which a larger negative charge increases the chance of gain of seed (27).

RESULTS

The human *AEG-1* gene contained 316 SNPs in the 3'UTR region. Based on the results of MirSNP for 316 3'UTR SNPs, 66 (20.9%) were determined to alter miRNAs binding (data shown in supplementary table 1). These variants could influence the function of *AEG-1* mRNA. Compared to other 3'UTR SNPs, analysis in the MirSNP software showed that 28 SNPs may significantly increase the putative miRNAs binding sites in this gene. MirSNP also predicted 21 mutated alleles that may decrease the seed length of several miRNAs targeting the related transcripts of the gene. Our evaluations in MirSNP also showed that the rs187728237

significantly affects the miRNA seed. We also found two gain of miRTSs and ten losses of miRTSs. Moreover, no target was found for the remaining 250 (79.1%) 3'UTR SNPs (Table 1). In MicroSNiPer, 67 (21.2%) SNPs were determined to affect the miRNA seeds, which were completely different from the results obtained by MirSNP. This algorithm identified 43 variations that might absolutely disrupt the miRNA seeds from seven (and more) to zero bp, while five other variants could exert an opposite effect. Overall, MirSNP and MicroSNiPer detected 143 and 290 different miRNAs that target *AEG-1*, respectively (Table 2).

Table1- Analysis of rs187728237 by MirSNP

MTDH/miRNA targets				
rsID	mRNA accession NO(NM)	miRNA	Energy change (Kcal/mol)	Effect by SNP on 3'UTR
	178812	hsa-miR-3609	Wild Type: -15.40 SNP Type: -16.30	Gain
	178812	hsa-miR-548ah-5p	Wild Type: -13.60 SNP Type: -14.60	Gain
	178812	hsa-miR-559	Wild Type: -17.50 SNP Type: 0.00	Loss
	178812	hsa-miR-548b-5p	Wild Type: -17.30 SNP Type: 0.00	Loss
	178812	hsa-miR-548c-5p	Wild Type: -15.60 SNP Type: 0.00	Loss
rs187728237 U --> C	178812	hsa-miR-548d-5p	Wild Type: -19.40 SNP Type: 0.00	Loss
	178812	hsa-miR-548h-5p	Wild Type: -14.60 SNP Type: 0.00	Loss
	178812	hsa miR-548w	Wild Type: -14.90 SNP Type: 0.00	Loss
	178812	hsa-miR-548o-5p	Wild Type: -15.60 SNP Type: 0.00	Loss
	178812	hsa-miR-548ab	Wild Type: -17.50 SNP Type: 0.00	Loss
	178812	hsa-miR-548am-5p	Wild Type: -15.60 SNP Type: 0.00	Loss
	178812	hsa-miR-548ay-5p	Wild Type: -18.60 SNP Type: 0.00	Loss

Table 2- List of miRNAs targeting rs80320514

rs80320514 [C/T]	
Seed length	**MicroRNA sets**
7-mer	hsa-miR-1321, hsa-miR-3151, hsa-miR-3689c, hsa-miR-3689a-3p, hsa-miR-3689b-3p, hsa-miR-4665-5p, hsa-miR-608, hsa-miR-4260, hsa-miR-202-3p, hsa-miR-570-5p, hsa-miR-3175, hsa-miR-30c-2-3p, hsa-miR-548ai, hsa-miR-92a-2-5p, hsa-let-7d-5p
8-mer	hsa-miR-4447, hsa-miR-4472, hsa-miR-5698, hsa-miR-92a-2-5p, hsa-miR-4787-5p, hsa-miR-4723-5p, hsa-miR-4787-5p
9-mer	hsa-miR-1275, hsa-miR-30c-1-3p

DISCUSSION

It is demonstrated that miRNAs, as posttranscriptional regulators, usually play a fundamental role in translation repression and target RNA destabilization by binding to the 3'UTR, 5'UTR or the coding region of the transcripts (28). Recent studies indicated that binding of miRNA to the 3'UTR region of transcripts is more effective in gene expression regulation (29). In addition, it has been suggested that miRNAs might interfere with numerous developmental processes, and could be considered as molecular biomarkers for several disorders (28).

Since preclinical studies in cancer biology are time consuming and extremely expensive, and require in vitro and in vivo animal experiments (30), in silico approaches prior to laboratory studies have been widely considered in recent investigations. Overexpression of the human *AEG-1* gene has been shown in malignant gliomas, breast carcinomas and melanomas (31), and we were able to identify putative miRTSs and affinity of candidate miRNAs in the presence of 3'UTR SNPs of this gene using the MicroSNiPer and MirSNP databases. Analysis by MirSNP indicted that rs187728237 might increase the affinity of two miRNAs and decrease the affinity of 10 other miRNAs to AEG-1 transcript. This variant might also increase the affinity of hsa-miR-3609 (energy charge in wild-type allele: 15.40 Kcal vs. mutant allele: 16.30 Kcal) and hsamiR-548ah-5p (energy charge in wild-type allele: 13.60 Kcal vs. mutant allele: 14.60 Kcal) (Table 1). Some studies reported overexpression of hsa-miR-3609 under endoplasmic reticulum stress (32) and in lung cancer (33). Considering the predicted target mRNAs of hsa-miR-548, functional enrichment analysis showed that the

miRNAgene family plays a major role in various biological processes and human diseases such as colorectal cancer, glioma and Alzheimer's disease (34). We found that rs187728237 might reduce the affinity of other miRNA variants including hsa-miR-559 and hsa-miR-548b-5p to the *AEG-1* gene transcript. It has been claimed that hsa-miR-548b-5p is significantly decreased in squamous cell carcinoma of the tongue (35). Transcriptional and post-transcriptional mechanisms are involved in regulation of ERBB2 protein expression. Hong Chen et al. claimed that 3'UTR of the *ERBB2* gene is the binding site for hsa-miR-559, indicating the possible involvement of this miRNA in *ERBB2* gene expression (36). Analysis of the 3'UTR variants of *AEG-1* gene by MicroSNiPer showed that rs80320514 might affect the binding site of 24 miRNAs (Table 2), including hsa-miR-1275 and hsa-miR-1321, which are thought to be involved in multiple sclerosis and pancreatic cancer, respectively (37, 38).

There is no direct approach for evaluating the accuracy of the predictions made by MirSNP and MicroSNiper, since the algorithms might have used different data sets. Although the predictions made by these tools were inconsistent for this subset of mutants, these AEG-1 variants should still be regarded as candidates for SNP screening.

It is suggested to conduct molecular and experimental studies to confirm our results, and further evaluate the possible regulatory functions of our predicted miRNA-related variants.

CONCLUSION

Based on our findings, it can be concluded

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that the 3'UTR SNPs located in the human AEG-1 gene may be within the miRTSs of the transcript, affecting the stability of putative miRNA-target interactions.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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