http://pmjournal.ir Original Article

Summer 2020, Volume 5, Issue 18 (10-13)

miR-146a rs2910164 polymorphism and lung cancer in a Tehran population

Niloofar Jahangir Sooltani[1*], Hossein Pakzad[2], Mohammad Ali Saremi[3]

[1] Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

[2] Department of biology, Tehran-east branch, Islamic azad university, Tehran, Iran.

[3] Personalized Medicine Research Center of AmitisGen, Tehran, Iran.

Corresponding author: Niloofar Jahangir Sooltani, Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran. xxxxxx

DOI: 10.22034/PMJ.2020.46380

Submitted: 2020/03/24
Accepted: 2020/06/08

Keywords:
Lung cancer
MicroRNA
RFLP-PCR genotype
Epithelial-mesenchymal transition

Abstract

Lung cancer is still the most common cancer worldwide in terms of the number of newly diagnosed cases and mortality rate. The expression of miR-146a was reduced in mesenchymal-like lung cancer cell lines. The overexpression of miR-146a induced a marked reduction of the mesenchymal marker and increase in the epithelial marker in lung cancer cell lines. The current study investigated the association of miR-146a genotypes and lung susceptibility in a Tehran population to determine the visibility of using RFLP-PCR genotype. The results revealed a significantly higher frequency of miR-146a CG and CC genotypes (p=0.01 and p=0.008, respectively) in patients compared with the control group. Those with the miR-146a GC and CC genotypes had an increased risk for developing lung cancer (OR=1.9; 95% CI: 1.1-3.3 and OR=4.1; 95% CI: 1.5-12.3, respectively). Moreover, the frequency rates of miR-146a CC genotype and C allele were significantly higher in patients than in the controls (p=0.006 and p=0.000, respectively)...

INTRODUCTION

Lung cancer (LC) is still the most common cancer worldwide in terms of the number of newly diagnosed cases and mortality rate (1). The incidence and mortality rates of LC vary greatly across different geographical locations and countries around the world. Most LC deaths occur in less developed countries. Tobacco smoke has been implicated as a primary causative agent in lung cancer (2). Other factors may also influence lung cancer risk, including age, gender, ethnicity, environment, occupational exposures, genetic and epigenetic changes. Data from recent studies has demonstrated the importance of regulatory mechanisms at the transcriptional level, such as gene regulation by small non-coding RNAs (microRNAs) (3). These mechanisms include the regulation of genes that mediate processes such as inflammation, the cell cycle, stress responses, differentiation, apoptosis, and invasion. In this context, research regarding the involvement of microRNAs in LC tumorigenesis is increasing as the search for new biomarkers and therapeutic targets continues (4). It is known that microRNAs are involved in the mechanisms of lung inflammation, epithelial-mesenchymal transition, and, consequently, in LC development and therapy response. The potential applications of microRNAs in cancer diagnostics and prognostics, and as therapeutic targets, have led to an increased interest in research in this area (5). The effects of microRNAs on cytokine signaling are based on transcription factors, cytokines, and modulators of cytokine signaling. In addition, cytokine signaling is crucial to the differentiation of many immune cells. Thus, the role of microRNAs in immune cell differentiation is based on the regulation of cytokine expression and the regulation of their downstream signaling components (6). Single nucleotide polymorphisms (SNPs) affecting miRNAs have also been a focus of recent interest. miRSNPs can affect several processes, including primary target gene transcription, pri-/pre-miRNA processing disturbance, and miRNA-mRNA interactions interruption (7). The dysregulation of miRNAs has been associated with various diseases, especially cancer, as it targets the genes which are involved in the regulation of cell proliferation and survival, DNA repair, and the immune response. The expression of miR-146a was reduced in mesenchymal-like lung cancer cell lines. The overexpression of miR-146a induced a marked reduction in the mesenchymal marker and increased the epithelial marker in lung cancer cell lines (8). Moreover, the overexpression of miR-146a suppressed lung cancer cell migration and invasion. Co-treatment with miR 146a and gefitinib showed a significant reduction in invasion into the resistant lung cancer cells

induced by Epithelial Mesenchymal Transition EMT (9). The expression of miR-146a was downregulated in advanced lung cancer tissue. The association between lung cancer and miR-146a rs2910164 polymorphisms has been intensively investigated, but the conclusions of the published studies are often in conflict. The current study investigated the association of miR-146a genotypes and lung susceptibility in a Tehran population to determine the viability of using these genotypes in identifying individuals who have increased lung cancer risk. The results might help the early diagnosis of lung cancer and may consequently improve prognoses.

METHODS AND MATERIALS

The case group included sixty lung cancer patients who were histopathologically diagnosed from chest and clinical oncology and 60 age- and gender-matched healthy subjects were enrolled in this study as control. Five milliliters of peripheral venous blood was collected in vacutainer tubes containing ethylenediaminetetraacetic acid. Genomic DNA was extracted from whole blood using the salting-out method. miR-146a rs2910164 genotyping was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism assay (RFLP). PCR conditions were an initial melting step at 94 °C for 5 min, followed by 30 cycles at 94 °C for 45 s, 63 °C for 30 s, and 72 °C for 45 s for the miR-146a gene. PCR products of miR-146a rs2910164 147 bp were digested by SacI restriction enzyme. For miR-146a, the presence of the G-allele resulted in no cleavage of the PCR product (147 bp); the C allele yielded two fragments of 122 and 25 bp. Data analysis was performed using SPSS software, and associations between miRNA SNPs and lung cancer risk were estimated by computing the odds ratios and their 95% confidence intervals. Results with a p-value 50.05 were considered statistically significant.

Results

The observed allele frequencies and genotype distributions of miR-146a agreed with the frequencies expected under Hardy-Weinberg equilibrium in both patients and controls. The current study revealed a significantly higher frequency of miR-146a CG and CC genotypes (p=0.01 and p=0.008, respectively) in patients compared with the control group. Those with the miR-146a GC and CC genotypes had an increased risk for developing lung cancer (OR=1.9; 95% CI: 1.1_3.3 and OR=4.1; 95% CI: 1.5_12.3, respectively). Furthermore, the frequency rates of miR-146a CC genotype and C allele were significantly higher in patients than in controls (p=0.006 and p=0.000, respectively).

Table 1. Comparisons of data using both alkaline and neutral comet assays

genotype	Case group	Control group	Sig.
GG	22(36.6%)	36 (60%)	0.04
GC	31 (51.6%)	20 (33.3%)	0.004
CC	7 (11.6%)	4 (6.6%)	0.01

DISCUSSION

Single nucleotide polymorphisms (SNPs) in miRNA genes and miRNA-associated pathways (miR-SNPs) have significant effects on gene expression and cellular processes by disrupting miRNA biogenesis and modulating miRNA-mRNA target interactions (10). MicroRNAs (miRNAs) are small (~22 nucleotides) noncoding RNAs that negatively regulate gene expression through complementary binding to the 3′ untranslated regions (3′UTRs) of their target messenger RNAs (mRNAs) (11). miRNAs are incorporated into Argonaute proteins in RNA-induced silencing complexes (RISCs), leading to target mRNA cleavage and degradation. SNPs in the miRNA regulatory pathways (miR-SNPs) that can be classified into the three categories of miRNA genes, miRNA biogenesis genes, and miRNA target genes can affect the transcription and processing precursor miRNA (pre-miRNA), modulate the affinity of miRNA-mRNA binding, abolish an existing binding site, or create abnormal binding sites (12). These inherited genetic miR-SNP variants in miRNA binding sites within the 3′UTRs of target genes can significantly contribute to cancer risk and adverse outcomes by regulating target gene expression and/or function (13). For example, an SNP in mature miR-196a2 was associated with lower survival rates in NSCLC patients. A pilot study showed that a haplotype of Drosha was significantly associated with shorter survival from lung cancer, and a SNP within the same haplotype was associated with reduced Drosha mRNA expression and resultant changes to global miRNA expression in lung adenocarcinoma tissues. The most exciting arena of miRNA SNPs is for those located within the miRNA binding sites of the 3′UTR of target genes (14). One of these conserved miRNAs is miR-146a, which is well-known for its important regulation of the immune response and inflammation (16). miR-146a is induced upon the activation of toll-like receptor 4 (TLR4) in the NF-κB-dependent signaling pathway, leading to the downregulation of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) (15). Recent studies

have demonstrated that the serum or plasma levels of miR-146a in septic patients were significantly decreased compared to those of normal controls and SIRS patients (16), suggesting that miR-146a may be significantly associated with sepsis. MiR-146a has been linked to osteoarthritis pathogenesis, as it has been shown to be an important negative regulator of immune responses through targeting two key genes, i.e. TNF Receptor-Associated Factor 6 (TRAF-6) and IL-1 Receptor-Associated Kinase 1 (IRAK-1). In advanced osteoarthritis, the downregulation of miR-146a expression is correlated with increased IRAK-1 and TRAF-6 levels and IL-1β mediated NF-κB signaling. Abnormal activation of NF-κB signaling induces inflammatory factors, such as interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), and catabolic mediators, such as matrix metalloproteinase-13 (MMP-13) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5), contributing to cartilage degradation and subsequent osteoarthritis progression. Previous studies have identified a functional SNP rs2910164 (G > C) located at the precursor molecule of miR-146a, which modulates miR-146a biogenesis and has been associated with the risk of many cancer types. The current findings suggest that the rs2910164 CG/GG genotypes in miR-146a are associated with a decreased risk for lung cancer. The association between miR-146a rs2910164 polymorphism and the risk for several malignancies was shown to be inconsistent by some results. The heterozygous genotype (CG) increased the risk of papillary thyroid cancer (17). Shen et al. (18) found that the C allele was associated with the increased risk of early onset familial breast and ovarian cancers. The C allele was also suggested to be a risk allele for gastric cancer in a Japanese study (19). The GG genotype was reported to correlate with an increased risk for hepatocellular carcinomas, esophageal squamous cell carcinoma, cervical cancer, and gastric cancer (20-23). Xu et al. (24) found that the CC genotype was associated with a decreased risk for prostate cancer. Jeon et al. (25) found that individuals carrying the CG or GG genotype were less likely to develop cancer compared with those carrying the CC genotype in a Korean population (25), which is similar to the present results. The present study provides evidence that polymorphism in miR-146a rs2910164 C>G might alter individual susceptibility to lung cancer by inhibiting miR-146a expression. Future larger studies with other ethnic populations and male lung cancer patients are required to confirm the current findings.

REFERENCE

1. Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., LortetTieulent, J., and Jemal, A. (2015) Jemal Global cancer statistics, 2012. CA Cancer J. Clin. 65,87108

2. Siegel, R.L., Miller, K.D., and Jemal, A. (2017) Cancer statistics, 2017. CA Cancer J. Clin. 67,730

3. Nieuwenhuis, L. and van den Brandt, P.A. (2019) Nut and peanut butter consumption and the risk of lung cancer and its subtypes: a prospective cohort study. Lung Cancer 128,5766

4. Filipowicz, W., Bhattacharyya, S.N., and Sonenberg, N. (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat. Rev. Genet. 9

5. Hu, Y., Yu, C.Y., Wang, J.L., Guan, J., Chen, H.Y., and Fang, J.Y. (2014) MicroRNA sequence polymorphisms and the risk of different types of cancer. Sci. Rep. 4, 3648

6. Esquela-Kerscher, A. and Slack, F.J. (2006) Oncomirs—microRNAs with a role in cancer. Nat. Rev. Cancer 6, 259269

7. Zhong, J.H., Li, J., Liu, C.F., Liu, N., Bian, R.X., Zhao, S.M., Yan, S.Y., and Zhang, Y.B. (2017) Effects of microRNA-146a on the proliferation and apoptosis of human osteoarthritis chondrocytes by targeting TRAF6 through the NF-kB signaling pathway. Biosci. Rep. 28, 37

8. Hummel, R., Hussey, D.J., and Haier, J. (2010) MicroRNAs: predictors and modifiers of chemo- and radiotherapy in different tumour types. Eur. J. Cancer 46, 298311

9. Volpe, M.V., Pham, L., Lessin, M., Ralston, S.J., Bhan, I., Cutz, E., and Nielsen, H.C. (2003) Expression of Hoxb-5 during human lung development and in congenital lung malformations. Birth Defects Res. Part A Clin. Mol. Teratol. 67, 550556

10. Vinci, S., Gelmini, S., Pratesi, N., Conti, S., Malentacchi, F., Simi, L., Pazzagli, M., and Orlando, C. (2011) Genetic variants in miR-146a, miR-149, miR-196a2, miR-499 and their influence on relative expression in lung cancers. Clin. Chem. Lab. Med. 49, 20732080

11. Xiao, S., Sun, S., Long, W., Kuang, S., Liu, Y., Huang, H., Zhou, J., Zhou, Y., and Lu, X. (2018) A meta-analytic review of the association between two common
SNPs in miRNAs and lung cancer susceptibility. Onco Targets Ther. 11, 24192427

12. World Medical Association (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA
310, 21912194

13. Sodhi, K.K., Bahl, C., Singh, N., Behera, D., and Sharma, S. (2015) Functional genetic variants in premiR-146a and 196a2 genes are associated with risk of lung cancer in North Indians. Future Oncol. 11, 215921731

14. Hu, Z., Chen, J., Tian, T., Zhou, X., Gu, H., Xu, L., Zeng, Y., Miao, R., Jin, G., Ma, H., Chen, Y., and Shen, H. (2008) Genetic variants of miRNA sequences and non small cell lung cancer survival. J. Clin. Invest. 118, 26002608

15. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real time quantitative PCR and 2-ct method. Methods 25, 402408

16. Shang, W., Pang, M., and Liu, Y. (2016) High resolution melting analysis of the correlation of the miRNA-196a2 gene polymorphism and lung cancer. BMU J. 2,8588

17. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci USA. 2008, 105:7269-7274.

18. Shen J, Ambrosone CB, DiCioccio RA, Odunsi K, Lele SB, Zhao H. A functional polymorphism in the miR-146a gene and age

of familial breast/ovarian cancer diagnosis. Carcinogenesis. 2008, 29:1963-1966.

19.Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, Yoshioka D, Yonemura J, Kamiya Y, Ishizuka T, Nakagawa Y, Nagasaka M, Iwata M, Yamada H, Hirata I, Arisawa T. Association study of common genetic variants in pre-microRNAs in patients with ulcerative colitis. J Clin Immunol. 2010, 31:69-73.

20. Xu T, Zhu Y, Wei QK, Yuan Y, Zhou F, Ge YY, Yang JR, Su H, Zhuang SM. A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis. 2008, 29:2126-2131.

21. Guo H, Wang K, Xiong G, Hu H, Wang D, Xu X, Guan X, Yang K, Bai Y. A functional variant in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. Fam Cancer. 2010, 9:599-603.

22. Yue C, Wang M, Ding B, Wang W, Fu S, Zhou D, Zhang Z, Han S. Polymorphism of the pre-miR-146a is associated with risk of cervical cancer in a Chinese population. Gynecol Oncol. 2011, 122: 33-37.

23. Zeng Y, Sun QM, Liu NN, Dong GH, Chen J, Yang L, Wang B. Correlation between pre-miR-146a C/G polymorphism and gastric cancer risk in Chinese population. World J Gastroenterol. 2010, 16:3578-3583.

24. Xu B, Feng NH, Li PC, Tao J, Wu D, Zhang ZD, Tong N, Wang JF, Song NH, Zhang W, Hua LX, Wu HF. A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. Prostate. 2010, 70:467-472.

25. Jeon HS, Lee YH, Lee SY, Jang JA, Choi YY, Yoo SS, Lee WK, Choi JE, Son JW, Kang YM, Park JY. A common polymorphism in pre-microRNA-146a is associated with lung cancer risk in a Korean population. Gene. 2014, 534:66-71.