Pre-microRNA Gene Polymorphisms and Risk of Cervical Squamous Cell Carcinoma

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ABSTRACT

Introduction: MicroRNAs (miRNAs) are short (~22 nucleotides) regulatory RNAs that can modulate gene expression and are aberrantly expressed in many diseases, including cancer. It has been suggested that, the presence of single nucleotide polymorphisms in precursor miRNAs (pre-miRNAs) can alter miRNA processing, expression and binding to target mRNA and represents another type of genetic variability, that can contribute to the susceptibility of human cancers.

Aim: The present study investigated the genetic variants in pre-miRNAs (hsa-miRNA-196a2 rs11614913 C/T, hsa-miRNA-499 rs3746444 T/C and hsa-miRNA-146a rs2910164 G/C) for their role in cervical cancer susceptibility.

Materials and Methods: The study comprised 164 controls and 184 patients of cervical cancer. The genotypic frequency of miRNA polymorphisms were determined by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-

RFLP) assay. Logistic regression was used for statistical analysis using SPSS Software version 15.0.

Results: Hsa-miRNA-499 rs3746444 T/C polymorphism showed a statistically significant association with considerable risk for cervical cancer at genotypes (CC, p=0.001, OR=4.801) and variant allele (p<0.001, OR=2.307). The miRNA 146a and miRNA 196a2 polymorphisms showed no association with cervical cancer. However, interaction of miRNA polymorphisms with smoking habit showed higher risk of cervical cancer with miRNA 196a2 polymorphism in patients with smoking but no significant modification in the risk of cervical cancer was seen for other polymorphisms.

Conclusion: The results of the present study demonstrate that, miRNA 499 T/C polymorphism is significantly associated with genetic susceptibility to cervical cancer and may have a role in its pathogenesis.

Keywords: Apoptosis, Cervical cancer, Single nucleotide polymorphism

INTRODUCTION

Cancer of the uterine cervix is the second most common cancer worldwide and is the leading cause of morbidity and mortality in the developing countries, including India. In India, the annual incidence of cervical cancer is approximately 132,000 with mortality rate of 74,000 [1]. Cervical cancer is a multistep process that involves the transformation of the normal cervical epithelium to a preneoplastic cervical intraepithelial neoplasia that is subsequently transformed to invasive cervical cancer [2]. Although, highrisk human papilloma viruses are associated with cervical cancer, human papilloma virus infection alone is not sufficient to induce the malignant transformation. Hence, other unidentified genetic alterations are likely involved. The identification of such genetic alterations would be of considerable importance for the screening and treatment of cervical cancer [3].

Recent studies have identified critical roles for miRNAs in a large number of biological processes, including development, differentiation, apoptosis and proliferation. It has been demonstrated that miRNAs suppress translation of protein coding genes or cleave target mRNAs destructively to induce their degradation by imperfect pairing with target mRNAs of protein coding genes, suggesting that miRNAs may regulate cellular gene expression at the transcriptional or post transcriptional level [4].

MiRNAs are small non coding RNAs that are 18 to 25 nucleotides in length; they regulate the stability or translational efficiency of target mRNAs. They are derived from primary transcripts (pri-miRNA) that are processed into hairpin precursors (pre-miRNAs) within the nucleus of the cell by the microprocessor complex, which includes the RNAse III enzyme Drosha [5]. Single Nucleotide Polymorphisms (SNPs) are the most frequent variation in the human genome,

occurring once every several hundred base pairs throughout the genome. Since the thermodynamics of RNA–RNA binding plays an essential role in miRNA interaction with target mRNA, it is expected that sequence variations such as SNPs at miRNA binding sites may affect the expression of miRNA targets. Consequently, a SNP located in the miRNA binding site of a miRNA target is likely to disrupt miRNA target interaction, resulting in the deregulation of target gene expression. Such SNP associated deregulation of the expression of an oncogenes or tumour suppressor gene might contribute to tumourgenesis [6]. Emerging evidence suggests that miRNAs might be involved in the pathogenesis of a variety of human cancers. The specific roles of miRNAs include the regulation of cell proliferation and metabolism developmental timing, cell death [7], haematopoiesis [8], neuron development, human tumourigenesis, DNA methylation and chromatin modification [9] Altered miRNA expression profiles have been reported in lung cancer, breast cancer, glioblastoma, hepatocellular carcinoma, papillary thyroid carcinoma and more recently, colorectal cancer [10].

Therefore, in the present study we evaluated the genetic variants in pre-miRNAs (hsa-miRNA-196a2 rs11614913 C/T, hsa-miRNA 499 rs3746444 T/C, and hsa-miRNA-146a rs2910164 G/C) for their role in cervical cancer susceptibility.

MATERIALS AND METHODS

This was a prospective case-control study, started from 1st January 2010 till 31st January 2012. Ethical committee clearance was taken from Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

A total of 348 females as study subjects were recruited, 184 cases of cervical cancer and 164 patients without any cervical pathology

as control. The cases were of similar ethnicity i.e., Northern Indians. The patients ranged from (22-80) years and all the cases enrolled in the study were biopsy proved of cervical cancer. Patients with any other associated cancer or those who had received prior chemoradiotherapy or had known any immunodeficiency disorder were excluded from the study. Informed consent was taken from all recruited patients. All the controls enrolled for the study were free from any cervical pathology and after physical examination they showed no symptoms of any debilitating disease. The patients were classified using guidelines set by International Federation of Gynaecology and Obstetrics Society [11], according to which there were 21 patients in Stage I, 63 in Stage II, 94 in Stage III, and 6 in Stage IV. Questionnaire was used for obtaining the demographic data and details such as patient's stage, smoking habit. Written informed consent was obtained from all the patients who agreed to participate in the study.

Blood samples (3.0 ml) from cases of cervical cancer and control subjects were collected in EthyleneDiamineTetraAceticAcid (EDTA) vials. Genomic DNA extraction from peripheral blood leucocytes was carried out using salting out method as described by Miller SA et al., Polymerase Chain Reaction (PCR) was conducted in a total volume of 10 µl with 0.5 pmol of each primer; 5'CATGGGTTGTGTC AGTGTCAGAGCT3'and 5' TGCCTTCTGTCTCCAGTCTTCCAA 3'; genomic DNA (100-150 ng); 10X Taq polymerase buffer (5 µl) and (1.5) units of Taq DNA polymerase (Bengaluru Genei, India) [12].

PCR conditions were as follows: Initial denaturation of 95°C for five minutes, 30 cycles of 95°C for 30 seconds, 67.6°C for 30 seconds and 72°C for 30 seconds; followed by final extension of 72°C for 10 minutes. PCR products were digested by restriction endonuclease SacI (MBI Fermentas) at 37°C for overnight and then analysed by 15% polyacrylamide gel electrophoresis using a 50 base-pair ladder.

STATISTICAL ANALYSIS

The sample size was calculated by using QUANTO software, version 1.1.0 (http://hydra.usc.edu/gxe). The sample size of 184 cases and 164 controls were adequate to give us a power of 80%. The χ^2 goodness of fit test was used for any deviation from Hardy–Weinberg equilibrium in controls and the Chi-square analysis was utilized to determine differences in genotype/allele frequencies. The age variable was expressed as mean±Standard Deviation (SD). Multivariate logistic regression analysis was used to estimate Odds Ratio (OR) and 95% Confidence Interval (CI) adjusted for age and gender to estimate the risk of GBC with miRNAs polymorphisms. Logistic regression was applied to estimate age-adjusted OR; tests of statistical significance were two-sided and taken as significant when p-value was less than 0.05. All statistical analyses were performed using Statistical Package for Social Sciences version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

The mean age of the 184 patients was 48.59±10.96 range (22-80), and that of controls was 47.44±9.8 mean±SD, range (20-79). Out of all the 184 cases enrolled in the study, 21 (11.4%) patients were in Stage I, 63 (34.2%) in Stage II, 94 (51.1%) in Stage III and 6 (3.3%) in Stage IV; 27.72% patients were found to be smokers and 72.28% were non smokers. All the controls enrolled in the present study were non smokers; they were healthy subjects devoid of any chronic debilitating disease in past or present.

The distribution of hsa-mir-196a2 C/T, hsa-mir-499 T/C and hsa-mir- 146a G/C genotypes and alleles in cervical cancer patients and control is shown in [Table/Fig-1]. The distribution of all the three miRNA variants in control samples were in HWE (Hardy Weinberg Equilibrium). The risk related to the three miRNA polymorphisms. hsa-miRNA-499 rs3746444 T/C polymorphism showed a statistically significant association with considerable risk for cervical

S. No	Geno-types/Alleles	Controls (n=164)	Patients (n=184)	OR* (95%CI) p-value
miRNA 146a				
1.	GG	84 (51.21%)	81 (44.02%)	Reference
2.	GC	72 (43.90%)	85 (46.19%)	1.218 (0.786-1.889)0.378
3.	CC	8 (4.87%)	18 (9.78%)	2.391 (0.982-5.818)0.055
	G	240 (73.17 %)	247 (67.11%)	Reference
	C	88 (26.82%)	121 (32.88%)	1.341 (0.966-1.861)0.079
miRNA 196a2				
1.	CC	21 (12.80%)	20 (10.86%)	Reference
2.	CT	81 (43.90%)	93 (50.54%)	1.233 (0.622-2.444)0.548
3.	TT	62 (37.80%)	71 (38.58%)	1.234 (0.610-2.495)0.588
	C	123 (37.5%)	133 (36.14%)	Reference
	T	205 (62.5%)	235 (63.85%)	1.070 (0.785-1.457)0.669
miRNA 499				
1.	TT	54 (32.9%)	26 (14.1%)	Reference
2.	CT	76 (46.34%)	78 (42.39%)	2.136 (1.214-3.757)0.008
3.	CC	34 (20.73%)	80 (43.47%)	4.801 (2.589-8.904)0.001
	C	144 (43.9%)	238 (64.6%)	Reference
	T	184 (56.0%)	130 (35.3%)	2.307 (1.619-3.131)0.001

[Table/Fig-1]: Distribution of hsa-miRNA gene polymorphisms.
*OR = odds ratio

miRNA gene polymorphisms	Controls	Early stages (I+II) n(%)	p-value	OR* (95%CI)
miRNA 146a				
GG	84 (51.2%)	37 (44.04)	-	Reference
GC	72 (43.9%)	38 (45.23)	0.204	1.440 (0.820-2.529)
CC	8 (4.87%)	9 (10.71)	0.067	2.55 (0.91- 7.14)
miRNA 196a2				
CC	21 (12.8%)	11 (13.09%)	-	Reference
CT	81 (43.9%)	46 (54.76%)	0.381	1.296 (0.726-2.314)
TT	62 (37.8%)	27 (32.14%)	0.688	1.193 (0.505-2.816)
miRNA 499				
CC	34 (20.73%)	37 (44.04%)	-	Reference
CT	76 (46.34%)	37 (44.04%)	0.008	2.984 (1.331 -6.691)
TT	54 (32.92%)	10 (11.90%)	0.001	6.488 (2.780 – 15.140)

[Table/Fig-2]: Association of miRNAs with early stages of cervical cancer.
*OR = odds ratio

cancer at genotypes (CC, p=0.001, OR=4.801) and variant allele (p<0.001,OR=2.307). However, miRNA 146a and miRNA 196a2 polymorphisms showed no association with cervical cancer.

[Table/Fig-2] shows the association of miRNAs with early stages of cervical cancer, here it was seen that hsa mir 146a (CC, p= 0.067, OR=2.55) showed risk with cervical cancer, while in hsa-mir-499 both heterogenotypes and variant genotypes (TC, p= 0.008, OR = 2.984) and (CC, p<0.001, OR= 6.488) showed significant risk with early stages of cervical cancer.

[Table/Fig-3] shows the comparison between early and advanced stages of cervical cancer to observe any effect of transition from early to advanced stages and their risk with miRNA. The results showed no risk for miRNAs in transition from early to advanced stages of cervical cancer.

Finally we also evaluated the effect of smoking, (bidi and cigarette), on the three polymorphisms in this study, hsa-miRNA-146a, hsa-miRNA-196a2, and hsa-miRNA-499. Interaction of miRNA

miRNA gene polymorphisms	Early stages (I + II) n (%)	Advanced stages (III +IV) n (%)	p-value	OR* (95%CI)
miRNA 146a				
GG	37 (44.04)	44 (44%)	-	Reference
GC	38 (45.23)	47 (47%)	0.338	0.739 (0.398-1.373)
CC	9 (10.71)	9 (9%)	0.044	0.329 (0.11-0.970)
miRNA 196a2				
CC	11 (13.09%)	9 (9%)	-	Reference
CT	46 (54.76%)	47 (47%)	0.141	0.623 (0.332-1.169)
TT	27 (32.14%)	44 (44%)	0.153	0.475 (0.171-1.319)
miRNA 499				
TT	10 (11.9%)	16 (16%)	-	Reference
CT	37 (44.04%)	41 (41%)	0.244	0.577 (0.229-1.454)
CC	37 (44.04%)	43 (43%)	0.290	(0.241-1.529)

[Table/Fig-3]: Association of early and advanced stages of cervical cancer with miRNA gene polymorphism.
*OR = Odds ratio

polymorphisms with smoking habit showed higher risk of cervical cancer with mir196a2 polymorphism in patients with smoking but no significant modification in the risk of cervical cancer was seen for other polymorphisms.

DISCUSSION

MiRNAs are an evolutionarily-conserved and abundant class of small non coding silencing RNAs, and some of them are expressed in limited developmental stages, in specific tissues or cells, which suggest that they may be involved in cell differentiation and maintenance of the properties of different cells. Mutations/ polymorphisms, mis-expression, or altered mature miRNA processing are likely to be pleiotropic and may contribute to cancer susceptibility and progression. Hsa-miRNA-196a2 and hsa-miRNA 146a, both having role in prostate cancer and refractory prostate cancer respectively are consistent with the hypothesis that miRNAs have substantial role in human cancers [13]. MiRNA has also been found to have role in other cancers, as seen in a study by Hu GZ et al., where hsa-miRNA-196a2 rs11614913:C/T and hsa-miRNA-499 rs3746444:T/C were associated with significantly increased risks for breast cancer [14]. All the three miRNAs polymorphisms evaluated in the present study are located in their corresponding 3p mature miRNA regions, and they may influence both the binding of target mRNAs to 3p mature miRNAs and pre-miRNA maturation of 5p and 3p miRNAs.

The SNPs rs3746444 and rs2910164 are located at the pre-miRNA regions of miRNA- 499 and miRNA-146a, respectively. The association between rs3746444 and rs2910164 significantly increased risk of breast cancer has also been reported, although this association was not supported with a larger sample size in different populations [15,16]. The SNP rs3746444 has also been investigated in lung cancer and coal worker's pneumoconiosis, but no association has been identified [17,18].

A recent study reported that miRNA-146a rs2910164 plays an important role in the risk and recurrence of bladder cancer, suggesting it may represent a biomarker for risk prevention and therapeutic intervention [19]. In a study on the above polymorphisms in Gall Bladder cancer (GBC) in India, it was found that in the hsa-miRNA-146a (rs2910164), hsa-mir-196a2 (rs11614913) and hsa-mir-499 (rs3746444) genes were associated with increased overall risk of developing GBC though the associations were non significant [20].

Recently a number of miRNAs have been evaluated in cervical cancer. MiRNA- 143 has been found to be down regulated, though it promoted apoptosis in cervical cancer patients as seen in Chinese population by Liu SS et al., [21]. Similar was the case with miRNA-372 which was down regulated in cervical cancer as by seen by Tian RQ et al., [22]. SNPs in pre-miRNAs such as, pre-miRNA-146a and pre-miRNA-499 have been found associated with cervical cancer risk in Chinese population by Yue C et al., and Zhou FH et al., [23,24]. In other cases, pre-miRNA-146a, pre-miRNA–499 and pre-miRNA 196a2 were found in bladder cancer and gall bladder cancers [25,26]. Chen J et al., identified six serum miRNAs: miRNA-1246, miRNA-20a, miRNA-2392, miRNA-3147, miRNA-3162-5p and miRNA-4484; and reported them as good predictor of Lymph node metastasis (LNM) with clinical value in early stage cervical squamous cell carcinoma [27]. Mi Y et al., found that rs 1131445 in the miRNA-135b binding site at IL-16 3'-UTR, can affect IL-16 protein expression by interfering with miRNA135b suppressive function, and is significantly associated with risk of cervical cancer [28].

In the present study, we observed that miRNA-499 variant alleles were significantly higher in cervical cancer cases. Hsa–miRNA - 499 showed significant allelic frequency. The significance of alleles was indicative of high risk, in patients of cervical cancer. The same has been observed in the Chinese population, the G allele of rs3746444 and G allele of rs2910164 may increase CSCC risk [29]. However miRNA-196a2 was not associated with cervical cancer risk in the present population, neither at genotypic or at allelic level. This no association in hsa-miRNA 196a2 might be due to the small sample size taken in this study or maybe this SNP has no role in cervical cancer susceptibility in the present population. In addition we classified the patients of cervical cancer into two groups based on early stages and those in advanced stages. The results showed that on comparing early and late stages of cervical cancer, no risk was found for the three polymorphisms, miRNA-499 showed significant increased risk with patients. In all the studies so far, we have not come across a single study in cervical cancer where the three SNPs have been evaluated in various stages of cervical cancer patients to see the effect of transition from early to later stages. The study thus shows that hsa-mir-499 has significant risk factor in role in cervical cancer.

Smoking is considered as cause of cervical cancer. In the present study, hsa-mir-196a2 had an association for risk with smoking in patients, though otherwise the polymorphism showed no association with cervical cancer patients.

Recently miRNAs have been recognized as important post transcriptional regulators of gene expression in mammals [30]. To date, >470 miRNAs have been identified in humans, and miRNAs are proposed to influence gene expression of >30% of proteincoding genes [31]. A miRNA gene is transcribed and processed initially into a precursor miRNA (pre-miRNA) that is ~100 bp in length and forms a stem-loop fold back structure [32]. The pre-miRNA is further processed into a Mature miRNA (MIR) that is ~22 bp long and binds to a specific target site on an mRNA to exert post transcriptional repression [33]. This is the first significant study on Indian population to the best of our knowledge to describe pre-miRNA SNPs with cervical cancer susceptibility.

CONCLUSION

The results of the present study imply that individual as well as combined genotypes of miRNA genes might influence cervical cancer. However, further studies are needed with larger sample size to strengthen the role of these miRNAs in cervical cancer.

LIMITATION

The study comprised a lower number of cases.

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REFERENCES

[1] Parkin DM, Ferlay J, Teppo L, Thomas DB. Cancer incidence in five continents. VIII, 2003 782.

[2] DiSaia PJ, Creasman WT. Pre-invasive disease of the Cervix. In: Clinical gynecologic oncology. 2002;14:01-34.

[3] Yoon JH, Lee JM, Namkoong SE. cDNA microarray analysis of gene expression profiles associated with cervical cancer. Cancer Res Treat. 2003;35(5):451-59.

[4] Lytle JR, Yario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′UTR as in the 3′UTR. Proc Natl Acad Sci USA. 2007;104(23):9667–72.

[5] Anita Q, Sofia N, Helena S. Non-coding RNAs: multi-tasking molecules in the cell. Int J Mol Sci. 2013;14(8):16010–39.

[6] Yu Z, Li Z, Jolicoeur N, Zhang L, Fortin Y, Wang E, et al. Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. Nucleic Acids. 2007;35:4535-41.

[7] Brennecke J, Stark A, Russell RB, Cohen SM. Bantam encodes a develop mentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hidin Drosophila. Cell. 2003;113:25-36.

[8] Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004;303:83-86.

[9] Bao N, Lye KW, Barton MK. MicroRNA binding sites in Arabidopsis class III HD-ZIP mRNAs are required for methylation of the template chromosome. Dev Cell. 2004;7:653-62.

[10] Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LA Jr, Sjoblom T, et al. The colorectal microRNAome. Proc Natl Acad Sci U S A. 2006;103:3687-92.

[11] Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. Int J Gynaecol Obstet. 2009;105(2):107-08.

[12] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.

[13] Ma X, Zhang T, Long B, Jiang D. Association between microRNA polymorphisms and cancer risk based on the findings of 66 case-control studies. PLoS One. 2013;8(11):e79584.

[14] Hu Z, Liang J, Wang Z, Tian T, Zhou X, Chen J, et al. Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mutat. 2009;30(1):79-84.

[15] He B, Pan Y, Cho WC, Xu Y, Gu L, Nie Z, et al The association between four genetic variants in MicroRNAs (rs11614913, rs2910164, rs3746444, rs2292832) and cancer risk: evidence from published studies. Plos One. 2012;7(11):e49032.

[16] Omrani M, Hashemi M, Eskandari-Nasab E, Hasani SS, Mashhadi MA, Arbabi F, et al. hsa-mir-499 rs3746444 gene polymorphism is associated with susceptibility to breast cancer in an Iranian population. Biomark Med. 2014;2:259-67.

[17] Tian T, Shu Y, Chen J, Hu Z, Xu L, Jin G, et al. A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev. 2009;18(4):1183-87.

[18] Wang M, Ye Y, Qian H, Song Z, Jia X, Zhang Z. Common genetic variants in pre-microRNAs are associated with risk of coal workers' pneumoconiosis. J Hum Genet. 2010;55(1):13-17.

[19] Wang M, Chu H, Li P, Yuan L, Fu G, Ma L, et al. Genetic variants in miRNAs predict bladder cancer risk and recurrence. The Journal of Cancer Research. 2012;10:1916-30.

[20] Srivastava K, Srivastava A, Mittal B. Common genetic variants in pre-microRNAs and risk of gallbladder cancer in North Indian population. J Hum Genet. 2010;55(8):495-99.

[21] Liu SS, Chan KY, Leung RC, Chan KK, Tam KF, Luk MH, et al. Prevalence and risk factors of Human Papillomavirus (HPV) infection in southern Chinese women : a population based study. PLoS One. 2011;5:e19244.

[22] Tian RQ, Wang XH, Hou LJ. MicroRNA-372 is down-regulated and targets cyclin-dependent kinase 2 (CDK2) and cyclin A1 in human cervical cancer, which may contribute to tumourigenesis. J Biol Chem. 2011;29:25556-63.

[23] Yue C, Wang M, Ding B, Wang W, Fu S, Zhou D, et al. Polymorphism of the pre-miR-146a is associated with risk of cervical cancer in a Chinese population. Gynecol Oncol. 2011;1:33-37.

[24] Zhao FH, Varanasi AP, Cunningham CA, Graubard BI, Hu SY, Chen F, et al. Tuberculosis and oncogenic HPV: potential co-infections in women at high-risk of cervical cancer in rural China. Asian Pac J Cancer Prev. 2011;12(6):1409-15.

[25] Bansal C, Sharma KL, Misra S, Srivastava AN, Mittal B, Singh US. Common genetic variants in pre-microRNAs and risk of breast cancer in the North Indian population. Ecancer medical science. 2011;8:473.

[26] Srivastava K, Srivastava A, Mittal B. Angiotensin I-converting enzyme insertion/deletion polymorphism and increased risk of gall bladder cancer in women. DNA Cell Biol. 2010;29(8):417-22.

[27] Chen J, Yao D, Li Y, Chen H, He C, Ding N, et al. Serum micro-RNA expression levels can predict lymph node metastasis in patients with early- stage cervical Squamous cell carcinoma. International Journal of Molecular Medicine. 2013;32(3):557-67.

[28] Mi Y, Wang L, Zong L, Pei M, Lu Q, Huang P. Genetic variants in micro-RNA target sites of 37 selected cancer-related genes and the risk of cervical cancer. Plos One. 2014;9(1):e86061.

[29] Zhou X, Chen X, Hu L, Han S, Qiang F, Wu Y. Polymorphisms involved in the miR-218 LAMB3 pathway and susceptibility of cervical cancer, a case-control study in Chinese women. Gynecol Oncol. 2010;117(2):287-90.

[30] Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature. 2005;438(7068):685–89.

[31] Liu W, Mao SY, Zhu WY. Impact of tiny miRNAs on cancers. World J. Gastroenterol. 2007;13(4):497–502.

[32] Lee Y, Ahn C, Han JJ, Choi H, Kim J, Yim J, The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003;425(6956):415–19.

[33] Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature. 2005;433(7027):769–73.

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