Pathology & Oncology Research (2020) 26:193–200
https://doi.org/10.1007/s12253-018-0412-x

ORIGINAL ARTICLE

Genetic Polymorphisms in miR-146a, miR-196a2 and miR-125a Genes and its Association in Prostate Cancer

Mohan Damodaran[1] · Solomon F. D. Paul[1] · Vettriselvi Venkatesan[1]

Received: 5 May 2017 / Accepted: 7 March 2018 / Published online: 29 March 2018

Abstract

The increase in incidence of prostate cancer in the Indian Population stresses the need to identify genetic markers for susceptibility and prognosis. Recent studies show that microRNAs play an important role in tumorigenesis by altering proliferation, differentiation and cell death. Gene polymorphisms not only in promoter region but also within miRNA gene have been shown to affect expression. The present study was aimed to analyze the role of miR-146a, miR-196a2 and miR-125a gene polymorphisms in prostate cancer. Genotyping of three SNPs rs73318382, rs57095329, rs2910164 in miRNA146a, rs11614913 in miR-196a2 and rs41275794, rs12976445, rs10404453 and rs1297533 in miR-125a was performed in 100 cases and 100 controls. Statistical analysis revealed the heterozygous AG genotype of the rs57095329 was significantly decreased in the cases when compared to the controls (OR-0.45, CI -0.24 to 0.85, *p* value-0.02) indicating an inverse association of this genotype with prostate cancer. Further the heterozygous CT of miR-196a2 (rs11614913) (OR-1.88, CI-1.06 to 3.35, p-0.02) and homozygous CC of miR-125a (rs12976445) (OR-2.55, CI -1.15 to 4.65, p-0.03) showed increased risk for prostate cancer. Combined analysis of all the genotypes revealed that the haplotype combination AGGCGTGG (OR = 0.09 at CI 95% (0.01–0.65) showed an inverse association with prostate cancer. Stratified analysis based on the age and tumor grade revealed no significant association.

Keywords microRNA · Prostate cancer · Genotyping · Haplotypes

Introduction

Prostate cancer is the second most frequently diagnosed cancer and leading cause of death among men [1]. In India, incidence of prostate cancer were increasing and the yearly average percentage change has ranged from 0.14–8.6 [2]. The molecular mechanisms underlying the development and progression of prostate cancer are unknown. A number of genes contribute to the development and progression of prostate cancer, among which miRNAs have powerful functions in gene regulation.

microRNAs (miRNAs) are evolutionarily conserved, small, endogenous, non coding RNA molecules of 18 to 24 nucleotide in length that function as post transcriptional gene regulators and also target the binding sites located in 3'untranslated regions of mRNAs [3]. miRNAs are negative gene regulators and map within fragile regions of chromosomes which are associated with various human cancers [4]. miRNA profiling in cancerous tissues have identified that they are involved in the key pathways related to proliferation, apoptosis and immune response and the deregulation of which is key to carcinogenesis [5]. The deregulated miRNAs may function as oncogenes or tumor suppressor genes and influence the initiation, progression and treatment outcomes in various cancers [6].

Since a specific miRNA has the potential to regulate the expression of hundreds of target mRNAs, SNPs in miRNAs may produce more significant functional consequences and represent an ideal candidate for disease prediction [7]. In the present study we chose to identify the association of SNPs in the pre-miRNAs on miR-125a, miR-146a and miR-196a2 with risk of prostate cancer.

miR-146 is involved in the regulation of inflammation and its expression is found to be up regulated by inflammatory factors such as interleukin 1 and tumor necrosis factor-alpha. Expression of miR146a down regulates a number of target genes which are involved in toll-like receptor pathways. Sun, et al., 2014 reported that miR-146a plays a suppressive role in prostate cancer through down-regulation of Rac1 [8].

✉ Vettriselvi Venkatesan
vettriselviv@yahoo.com

[1] Department of Human Genetics, Sri Ramachandra University, Chennai 600116, India

Earlier study by Lin et al. 2008 reported that miR-146a may function as a tumor-suppressor gene in modulating HA/ROCK1-mediated tumorigenecity [9].

miR-196a2 consists of two types of mature miRNAs namely miR-196a-5p and miR-196a-3p both of which are processed from the same stem-loop [10]. Earlier studies have reported alterations in miRNA expression and associated 137 cancer-related transcripts after the introduction of pre-miR-196a-C vector [11]. The rs11614913 polymorphism located in the mature sequence of miR-196a-3p could lead to less efficient processing of the miRNA precursor to its mature form and reduce the capacity to regulate target genes [12]. Hoffman et al. [11] reported that miR-196a2 rs11614913 not only influences the levels of mature miR-196a, but also has a phenotypic effect on target gene expression [13].

Among the most important miRNA families, miR-125 family has been reported to be implicated in a variety of carcinomas and other diseases as either repressors or promoters. MiR-125a was demonstrated to control major cellular processes in a number of cancer cells. Over-expression of miR-125a impaired migration and invasion of breast cancer cells [14]. Expression of miR-125a resulted in inhibition of proliferation and metastases in hepatocellular carcinoma cells [15]. Studies have also reported the role of miR-125a in the process of invasion and migration in gastric and lung cancer cells [16].

Reported evidence clearly suggests that miR-125a, miR-146a and miR-192-a2 microRNAs play a critical role in tumor invasion and metastasis. Though many studies have identified the role of these microRNAs in various cancers, only limited literature exist for the same prostate cancer. In view of the critical role of these miRNAs and their SNPs not only influence mature miRNA expression, but also affects target gene expression. Therefore, the present study was designed to investigate the role of SNPs in the miR-125a, miR-146a and miR-196a2 prostate cancer in a South Indian population.

Materials and Methods

Study Population

Histologically confirmed prostate cancer samples ($n = 100$) were collected from Sri Ramachandra Medical centre, Chennai. The age of the patients range from 52 years to 75 years and the mean age were 67 years. Relevant clinical and pathological data were collected from all the patients. At the time of diagnosis, the PSA levels of all the individuals were recorded. The PSA levels ranging from 4.0 ng/mL were considered for study. Pathological grading of the tumors represented as Gleason scores (GS) was obtained by the histopathological examination. Patients with secondary tumor development were excluded from study. Controls ($n = 100$) with no history of cancer, and age ranging from 60 years to 85 years with a mean age of 70 years were recruited. Ethical clearance was obtained from the Institutional Ethics Committee, Sri Ramachandra University. Informed consent was obtained from each subject at the time of enrollment.

Genotyping

About 2 ml of blood was collected from all the subjects in EDTA vacutainer. Genomic DNA was extracted from peripheral blood using QIAamp DNA mini kit according to the manufacturer's protocol and stored at −20 °C until further processing. In this study 4 sets of primers were used, primer 1 was chosen to amplify the promoter region of the miR-146a (promoter, nt-749 to nt −259) [17], the primer 2 to amplify the pre-miR-146a [17]. Primer 3 was chosen to amplify pre-miR-196a2 [18]. Primer 4 was chosen to amplify pri-miR-125a [19].

PCR was carried out in a total reaction volume of 20 μl containing 100 ng of genomic DNA, 30pM of each primer(forward and reverse), 1X PCR buffer, 200 μM dNTP and 3 units of Taq DNA polymerase. The PCR cycling conditions were carried out as follows: 98 °C for 2 min, 56 °C for 5 s, 72 °C for 35 s; 28 cycles and final extension 72 °C for 5 min. Genotyping of miR-146a and miR-125a was performed by direct dye terminator of sequencing. The sequencing data was analyzed using Seqscape software v2.5. miR-196a2 rs11614913 C/T genotyping was performed by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) genotyped using the restriction enzyme *MspI,* which yields the product size 24 bp and 125 bp for genotype CC, 149 bp for genotype TT and 149 bp,125 bp and 24 bp for heterozygous CT [18]. Representative samples from each genotype were sequenced to confirm the genotypes.

Statistical Analysis

Statistical analysis was performed using the SNPstats online tool [20]. The expected genotype and allele frequencies for the observed variations were calculated for cases and controls. These frequencies were tested for Hardy-Weinberg equilibrium. The association of SNPs in miR-146a, miR-196a2 and miR-125a with prostate cancer was evaluated using odds ratio at 95% confidence interval (CI). The association of the genotypes of all the SNPs with clinical parameters including tumor grade, PSA and age were calculated using odds ratio at 95% confidence interval (CI). P values <0.05 were considered statistically significant.

Result

The association of miR-146a, miR-196a2 and miR-125a gene polymorphisms with prostate cancer estimated using odds ratio

Table 1 Association analysis of miR-146a, miR-196a2 and miR-125a gene polymorphisms with prostate cancer patients and controls estimated using odds ratio (OR) at 95% confidence interval (CI) and *P*-value

Gene	Genotype	Control (n = 100)	Case (n = 100)	OR (95% CI)	P-value
miR-146a	rs73318382				
	AA	77(77%)	73 (73%)	Ref	
	AC	20(20%)	18(18%)	0.9 (0.4–1.9)	0.19
	CC	3 (3%)	9 (9%)	3.16(0.82–12.15)	0.14
	AC + CC	23 (23%)	27 (27%)	1.24 (0.65–2.35)	0.51
	AA+AC	97(97%)	91 (91%)	Ref	
	CC	3 (%)	9 (9%)	3.20(0.84–12.18)	0.068
	A allele frequency	0.87	0.82		
	C allele frequency	0.13	0.18		
	rs57095329				
	AA	63 (63%)	79 (79%)	Ref	
	AG	34 (34%)	19 (19%)	0.45 (0.23–0.86)	0.02
	GG	3 (3%)	2 (2%)	0.53 (0.09–3.28)	0.81
	AG + GG	37(37%)	21 (21%)	0.45(0.24–0.85)	0.012
	AA+AG	66 (66%)	98 (98%)	Ref	
	GG	3 (3%)	2(2%)	0.44(0.07–2.7)	0.65
	A allele frequency	0.8	0.88		
	G allele frequency	0.2	0.12		
	rs2910164				
	GG	51(51%)	52 (52%)	Ref	
	CG	39(39%)	38 (38%)	0.96(0.53–1.72)	0.99
	CC	10(10%)	10(10%)	0.98(0.38–2.56)	1
	CG + CC	49(49%)	48 (48%)	0.96(0.55–1.67)	1
	GG + CG	90(90%)	90 (90%)	Ref	
	CC	10 (10%)	10 (10%)	1.00 (0.40–2.52)	1
	G allele frequency	0.7	0.71		
	C allele frequency	0.3	0.29		
miR-196a	rs11614913				
	CC	47 (47%)	32 (32%)	Ref	
	CT	36(36%)	51 (51%)	2.08(1.12–3.87)	0.02*
	TT	17 (17%)	17 (17%)	1.47(0.65–3.30)	0.46
	CT + TT	53(53%)	68(68%)	1.88(1.06–3.35)	0.04*
	CC + CT	83(83%)	83(%)	Ref	
	TT	17(17%)	17(17%)	1.00(0.48–2.09)	1
	C allele frequency	0.65	0.57		
	T allele frequency	0.35	0.42		
miR-125a	rs41275794				
	G/G	100	100		
	rs12976445				
	T/T	37(37%)	28(28%)	Ref	
	C/T	48(48%)	43(43%)	1.18(0.62–2.25)	0.72
	C/C	15(15%)	29(29%)	2.55(1.16–5.65)	0.03*
	C/T + C/C	63(63%)	72(72%)	1.51(0.83–2.74)	0.22
	T/T + C/T	85(85%)	71(71%)	Ref	
	C/C	15(15%)	29(29%)	2.31(1.15–4.65)	0.026*
	T allele frequency	0.61	0.5		
	C allele frequency	0.39	0.5		
	rs10404453				
	G/G	100	100		
	rs1297533				
	G/G	100	100		

* p-value <0.05 is significant.; Ref: indicates the reference genotype (wild type)

NS, Not significant

at 95% confidence interval is represented in Table 1. rs57095329, rs2910164, rs41275794, rs12976445, rs10404453, rs1297533 were found to follow Hardy-Weinberg equilibrium while rs73318382 in case group and rs11614913 in control group did not follow Hardy-Weinberg Equilibrium. In rs57095329, the heterozygous AG genotype was found to be significantly higher in controls compared to cases with OR = 0.45, 95%CI = 0.23–0.86 and $P = 0.043$. Similar result was observed in the dominant model (AA vs AG + GG) with OR = 0.43, 95%CI = 0.24–0.85 and $P = 0.85$.

Table 2 Haplotype analysis of miR-146a, miR-196a and miR-125a gene polymorphisms between prostate cancer patients and controls

S.No	rs73318382	rs57095329	rs2910164	rs11614913	rs41275794	rs12976445	rs10404453	rs1297533	Frequency for controls	Frequency for cases	OR (95%CI)	P –Value
1	A	A	G	C	G	T	G	G	0.22	0.24	1.00	
2	A	A	G	C	G	C	G	G	0.13	0.12	0.94 (0.4–2.24)	0.89
3	A	A	G	T	G	T	G	G	0.1	0.1	1.14 (0.47–2.75)	0.77
4	A	A	G	T	G	C	G	G	0.04	0.13	3.11 (0.88–10.92)	0.079
5	A	A	C	T	G	C	G	G	0.05	0.07	1.59 (0.4–6.37)	0.51
6	A	A	C	C	G	T	G	G	0.07	0.05	1.01 (0.34–3.0)	0.98
7	A	A	C	C	G	C	G	G	0.07	0.04	0.33 (0.07–1.51)	0.15
8	A	A	C	T	G	T	G	G	0.04	0.03	0.53 (0.14–2.04)	0.36
9	A	G	G	C	G	T	G	G	0.06	0.01	0.09 (0.01–0.65)	0.019
10	C	G	G	T	G	T	G	G	0.03	0.012	4.44 (0.42–46.92)	0.22
11	C	G	C	C	G	C	G	G	0.008	0.03	0.52(0.1–2.63)	0.43
12	C	G	G	C	G	T	G	G	0.02	0	0.42 (0.06–3.07)	0.39
13	C	A	G	C	G	T	G	G	0.01	0.0096	0.39 (0.02–6.02)	0.5
14	C	G	G	T	G	C	G	G	0.016	0.015	1.55 (0.16–15.26)	0.71

* P-value <0.05 is significant

This indicates that G allele has inverse effect on prostate cancer. rs73318382 and rs2910164 in the miR-146a did not show any association with prostate cancer. The heterozygous CT genotype of miR-196a2(rs11614913) was found to be statistically significant in cases when compared to controls, with an OR of 2.08 and 95% CI of 1.27 to 3.87 (*p* value-0.02). Similar results were observed in the dominant model with an OR of 1.88 and 95% CI of 1.06 to 3.35 (p value-0.04); thereby indicating an increased risk of prostate cancer. In miR-125a gene, 4 SNP's were analyzed (rs41275794, rs10404453, rs1297533 and rs12976445) out of which 3 SNP's (rs41275794, rs10404453 and rs1297533) showed only single genotype GG (monomorphic) and the rs12976445 polymorphism in miR-125a revealed the homozygous CC genotype to be significantly higher in cases with OR = 2.55, 95%CI = 1.16–5.65,*P* = 0.03. Similarly, the recessive model (TT + TC vs CC) was found to be significantly higher in cases with OR = 2.31 and 95% CI = 1.15–4.65, *P* = 0.026. Therefore, CC mutant genotype was found to increase the risk of prostate cancer.

Haplotype analysis of miR-146a, miR-196a2 and miR-125a gene polymorphisms in cases and controls are represented in Table 2. A total of 14 haplotype combinations were observed among which the haplotype AGGCGTGG was found to be higher in controls when compared to cases with an OR of 0.09 and 95% CI 0.01–0.65.

The allele frequencies observed in the present study were compared with the frequencies available in the HapMap data. Our results revealed that rs2910164 has higher C allele frequency when compared with other populations such as European, African, American and Asian. Moreover, rs11614913 and rs12976445 showed similar allele frequencies with American population. The allele frequencies of rs10404453 did not show any significant difference among other populations.

Association with Clinical Parameters

The genotypes of the polymorphisms of miRNAs miR-146a, miR-196a2 and miR-125a were compared with the clinical parameters including Gleason Score, age at diagnosis. For Gleason score, the patients were grouped into two categories (Low grade < 7 (less aggressive) and high grade ≥ 7 (more aggressive)). No significant difference was observed in genotype frequencies between patients in low and high tumor grade. Figure 1 represents the distribution of the genotypes based on tumor grade. No significant difference was observed between the genotype frequency and PSA at diagnosis.

Discussion

In this paper we present a case-control study of seven polymorphisms from 3 miRNAs miR-146a, miR-196a2 and miR-125a. miRNAs are differentially expressed in different types of cancer [21]. miRNA has multiple targets and they function by degrading target mRNA. The presence of single nucleotide polymorphisms in the primary-miRNAs affect the maturation process of miRNA thereby hindering binding efficacy of miRNA to its target, leading to cancer susceptibility [22]. The over expression of miR-146a has been identified in cancer cell lines and tumor tissues. The miR-146a polymorphism rs2910164, is located on chromosome 5q33 at the 60th nucleotide position in the seed region of pre-miR-146a [23], meanwhile rs 57,095,329 is located in promoter region at −386 nucleotide position and rs73318382 is located in promoter region at −690 nucleotide position. The variant rs2910164 changes G:U to C:U leading to mispairing in the hairpin of miR-146a and decreases the efficiency of processing pre-miRNA to mature miRNA, thereby represses the expression

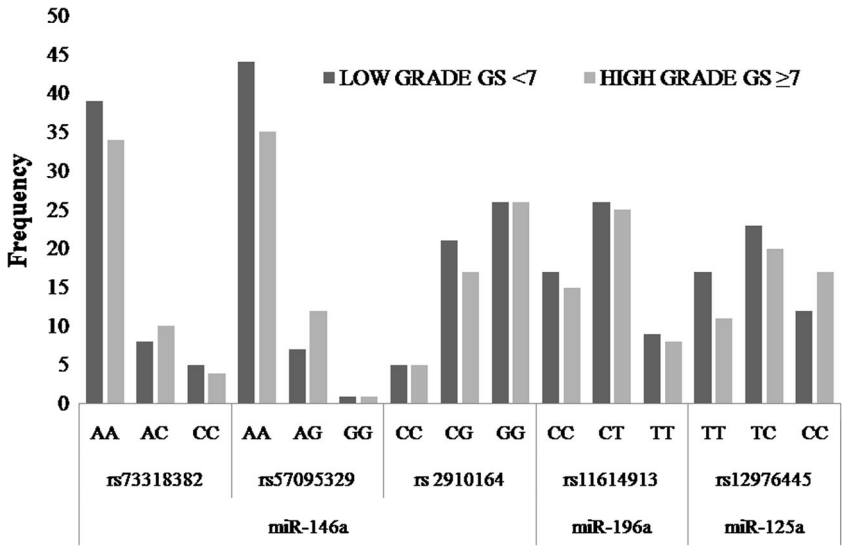

Fig. 1 Distribution of genotypes based on tumor grade

of its target genes [24]. Studies carried out in papillary thyroid carcinoma shows altered expression of miR-146a and implicated as an important link between inflammation and carcinogenesis [25, 26]. A loss of miR-146a function was reported in hormone refractory prostate cancer [27]. Functional analysis of Castration-resistant prostate cancer and androgen dependent prostate cancer showed over expression of miR-146a in androgen-independent cell lines, inhibit cell growth, colony formation, migration and inhibit the expression of MMP2 gene responsible for tumor progression [28].

The genotypes of rs73318382 and rs2910164 polymorphisms did not reveal any significant difference among controls and cases. A study in North Indian population on the role of gene polymorphisms in miRNA revealed no association of rs2910164 with prostate cancer [29]. Our study results are in agreement with the North Indian study. Similar study on the role of rs2910164 G > C polymorphism in Chinese Han population reported that subjects carrying CC genotype showed reduced risk of prostate cancer by 0.65 folds [30]. No such association was observed in the present study.

miR-196a-1 located on chromosome 17, miR-196a2 located on chromosome 12 and miR-196b located on chromosome 7 belongs to the family of miR-196. The sequence of miR-196a differs from miR-196b by one nucleotide. The miR-196a2 polymorphism rs11614913 is located in the mature

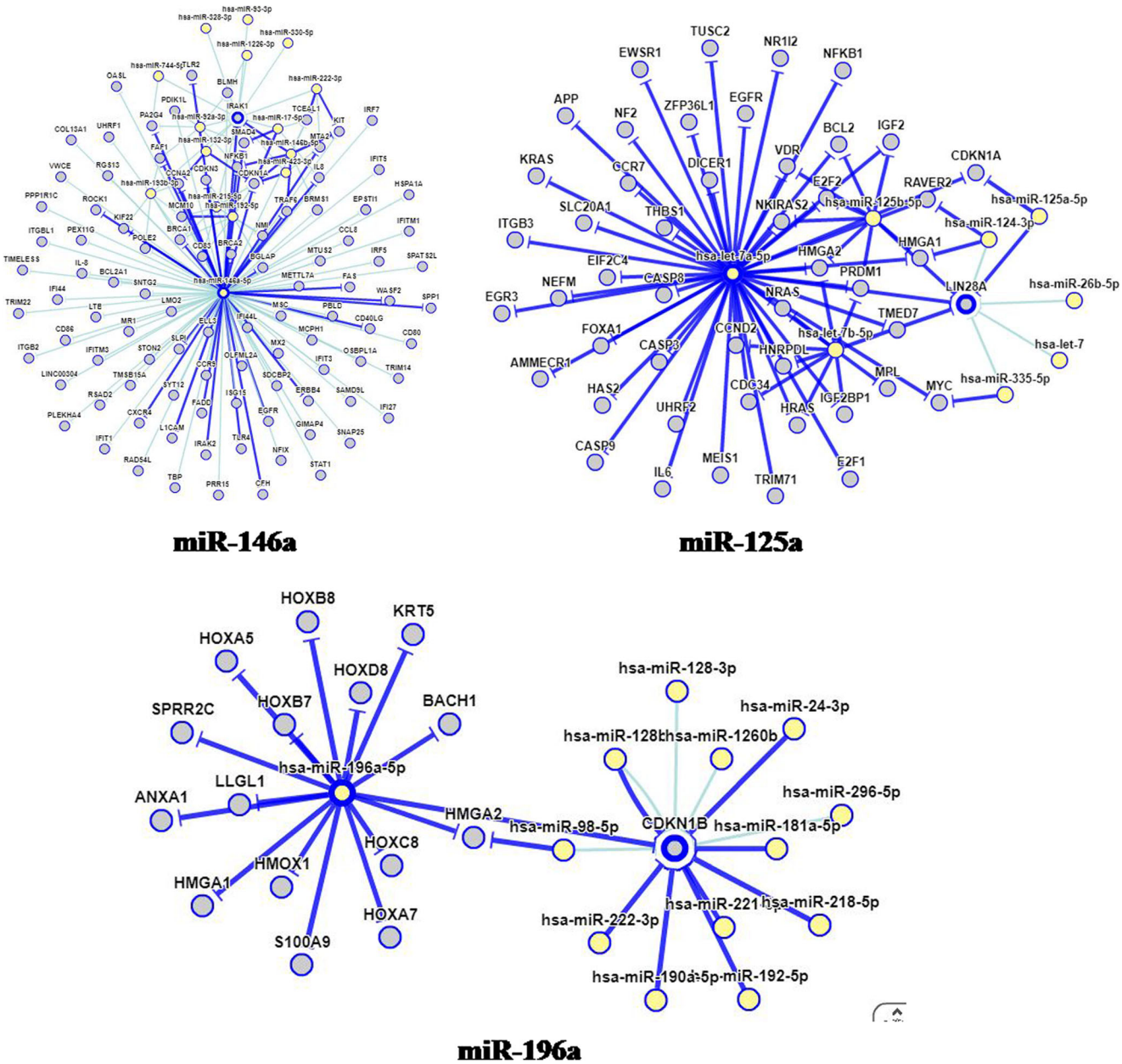

Fig. 2 Predicted gene targets for miR-146a, miR-125a and miR-196a

sequence of miR-196a-3p (passenger strand), which affects the processing of pre-miRNA to its mature form and also the capacity to regulate target genes [11].

Our study revealed that the heterozygous CT genotype (OR = 2.08, CI = 1.12–3.87) and the dominant model (OR = 1.88, CI = 1.06–3.35) of pre-miR-196a polymorphism rs11614913 were found to be significantly higher in Prostate cancer patients compared to controls indicating increased risk of prostate cancer susceptibility. This is in concordance with study on North Indian population in prostate cancer [29] and similar results were observed in a study on colorectal cancer in Chinese population [31]. To the best of our knowledge, this is the first study in South Indian population investigating the association of miR-146a and miR-196a2 polymorphisms with Prostate Cancer susceptibility.

miR-125 family consist of miR-125a, miR-125b-1 and miR-125b-2. miR-125a is located at 19q13 while miR-125b is located at 11q23(miR-125b-1) and 21q21(miR-125b-2) [32]. The polymorphism rs12976445 revealed that homozygous CC genotype and the recessive model (TT + TC vs CC) were associated with risk of prostate cancer. This is the only study which shows association of rs12976445 with prostate cancer. miR-125a has been reported to be down-regulated in breast cancer biopsy and functions as tumor suppressors [14, 33, 34] by targeting the ETS1 gene through ERBB2 and ERBB3 pathway [33].

The stratified analysis did not show any association of genotypes with clinical parameters. Moreover further studies on the functional role of miR-146a, miR-196a2 and miR-125a will help in understanding the pathogenesis and progression of prostate cancer.

The miRNAs analyzed in the present study revealed a significant association with prostate cancer, to gain insight into the biological role of each miRNA, we performed a target prediction analysis to identify the full repertoire of its mRNA targets using miRTarBase, miRwalk and gprofiler. Figure 2 represents the target genes for each of the microRNAs, the results clearly indicates a highly complex set of interactions between miRNAs and target mRNAs. Methodologically studying each miRNAs and mRNAs in several models will help to clarify their roles in prostate cancer. A better understanding of these pathways will bring us yet closer to an understanding of the molecular mechanism underlying this disease and hopefully to novel approaches for detection and therapy.

Conclusion

The present study showed that miR-146a polymorphism rs57095329 had an inverse association with prostate cancer. More over miR-196a2 (rs11614913) and miR-125a (rs12976445) polymorphism showed a positive association with risk of prostate cancer.

Clinical Significance

microRNAs are small RNA fragments that regulate gene expression via translational repression. Studies have shown that polymorphism affects the regulatory capacity of miRNAs by influencing miRNA processing and/or miRNA-mRNA interactions. While miRNAs are involved in various physiological mechanisms, a number of studies have provided evidence that polymorphisms in miRNA may be related to increase patient susceptibility, patient prognosis and survival. According to the various functions, it is reported that high expression of miRNA causes superior survival and low expression of miRNA can yield opposite result. Based on the above evidence we adopted a case-control study design to analyze the relationship between miRNA146a, miRNA-196a and miRNA-125a gene polymorphisms and prostate cancer. We also performed the combined analysis of the miRNA genotypes with age, clinical stage and patient susceptibility to prostate cancer. The results of our study indicate that miR-146a rs 57,095,329 variant showed an inverse association with prostate cancer, However the miR-196a (rs11614913) and miR-125a (rs12976445) variants revealed an increased risk for prostate cancer with an odds ratio of 2.55 (CI 1.15 to 4.65,p-0.03). Although we observed evidence that these miRNAs are related to prostate cancer susceptibility, additional laboratory experiments and functional assays are required to confirm the effects of these gene polymorphisms on prostate cancer.

Funding This work is a part of the ongoing funded project by Department of Biotechnology, Government of India 6242-P58/RGCB/PMD/DBT/VVSL/2015.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90
2. Lalitha K, Suman G, Pruthvish S, Mathew A, Murthy NS (2012) Estimation of time trends of incidence of prostate canner-an Indian scenario. Asian Pac J Cancer Prev 13(12):6245–6250
3. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. Science 294(5543):853–858
4. Esquela-Kerscher A, Slack FJ (2006) Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer 6(4):259–269
5. Cho WC (2007) OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer 6(1):60
6. Sun J, Lu H, Wang X, Jin H (2013) MicroRNAs in hepatocellular carcinoma: regulation, function, and clinical implications. Sci World J 2013:1–14
7. Lu J, Gu H, Tang Q, Wu W, Yuan B, Guo D, Hu L (2016) Common SNP in hsa-miR-196a-2 increases hsa-miR-196a-5p expression and predisposes to idiopathic male infertility in Chinese Han population. Sci Rep 6
8. Sun Q, Zhao X, Liu X, Wang Y, Huang J, Jiang B et al (2014) miR-146a functions as a tumor suppressor in prostate cancer by targeting Rac1. Prostate 74(16):1613–1621
9. Lin SL, Chiang A, Chang D, Ying SY (2008) Loss of mir-146a function in hormone-refractory prostate cancer. RNA 14(3):417–424

10. Chen C, Zhang Y, Zhang L, Weakley SM, Yao Q (2011) MicroRNA-196: critical roles and clinical applications in development and cancer. J Cell Mol Med 15(1):14–23
11. Hoffman AE, Zheng T, Yi C, Leaderer D, Weidhaas J, Slack F et al (2009) microRNA miR-196a-2 and breast cancer: a genetic and epigenetic association study and functional analysis. Cancer Res 69(14):5970–5977
12. Hu Z, Liang J, Wang Z, Tian T, Zhou X, Chen J, Miao R, Wang Y, Wang X, Shen H (2009) Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mutat 30:79–84
13. Wang J, Wang Q, Liu H, Shao N, Tan B, Zhang G, Cheng Y (2012) The association of miR-146a rs2910164 and miR-196a2 rs11614913 polymorphisms with cancer risk: a meta-analysis of 32 studies. Mutagenesis 27(6):779–788
14. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC (2007) Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. J Biol Chem 282(2):1479–1486
15. Bi Q, Tang S, Xia L, Du R, Fan R, Gao L, Nie Y (2012) Ectopic expression of MiR-125a inhibits the proliferation and metastasis of hepatocellular carcinoma by targeting MMP11 and VEGF. PLoS One 7(6):e40169
16. Ninio-Many L, Grossman H, Levi M, Zilber S, Tsarfaty I, Shomron N et al (2014) MicroRNA miR-125a-3p modulates molecular pathway of motility and migration in prostate cancer cells. Oncoscience 1(4):250
17. Luo X, Yang W, Ye DQ, Cui H, Zhang Y, Hirankarn N, Qian X, Tang Y, Lau YL, De Vries N, Tak PP (2011) A functional variant in microRNA-146a promoter modulates its expression and confers disease risk for systemic lupus erythematosus. PLoS Genet 7(6): e1002128
18. Zhou B, Rao L, Peng Y, Wang Y, Chen Y, Song Y, Zhang L (2010) Common genetic polymorphisms in pre-microRNAs were associated with increased risk of dilated cardiomyopathy. Clin Chim Acta 411(17):1287–1290
19. Williams, A. E., Perry, M. M., Moschos, S. A., Larner-Svensson, H. M., & Lindsay, M. A. (2008). Role of miRNA-146a in the regulation of the innate immune response and cancer
20. Solé X, Guinó E, Valls J, Iniesta R, Moreno V (2006) SNPStats: a web tool for the analysis of association studies. Bioinformatics 22(15):1928–1929
21. Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D. … & Downing, J. R. (2005). MicroRNA expression profiles classify human cancers nature, 435(7043), 834–838
22. Lv M, Dong W, Li L, Zhang L, Su X, Wang L, Gao L, Zhang L (2013) Association between genetic variants in pre-miRNA and colorectal cancer risk in a Chinese population. J Cancer Res Clin Oncol 139(8):1405–1410
23. Zhang X, Gu Y, Liu X, Yu Y, Shi J, Yu Q, Sun H, Kanu JS, Zhan S, Liu Y (2015) Association of pre-miR-146a rs2910164 polymorphism with papillary thyroid cancer. Int J Endocrinol 2015:1–9
24. Chae YS, Kim JG, Lee SJ, Kang BW, Lee YJ, Park JY, Jeon HS, Park JS, Choi GS (2013) A miR-146a polymorphism (rs2910164) predicts risk of and survival from colorectal cancer. Anticancer Res 33(8):3233–3239
25. Williams AE, Perry MM, Moschos SA, Larner-Svensson HM, Lindsay MA (2008) Role of miRNA-146a in the regulation of the innate immune response and cancer. Biochem Soc Trans 36:1211–1215
26. Kati PP, Minja JP, Waltering KK et al (2007) microRNA expression profiling in prostate cancer. Cancer Res 67(13):6130–6135
27. Shi-Lung L, Angela C, Donald C, Shao-Yao Y (2008) Loss of miR-146a function in hormone-refractory prostate cancer. RNA 14:417–424
28. Xu B, Wang N, Wang X, Tong N, Shao N, Tao J, Li P, Niu X, Feng N, Zhang L, Hua L, Wang Z, Chen M (2012) miR-146a suppresses tumor growth and progression by targeting EGFR pathway and in a p-ERK- dependent manner in castration-resistant prostate cancer. Prostate 72(11):1171–1178
29. George GP, Gangwar R, Mandal RK, Sankhwar SN, Mittal RD (2011) Genetic variation in microRNA genes and prostate cancer risk in north Indian population. Mol Biol Rep 38(3):1609–1615
30. Xu B, Feng NH, Li PC, Tao J, Wu D, Zhang ZD, Tong N, Wang JF, Song NH, Zhang W, Hua LX (2010) A functional polymorphism in pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. Prostate 70(5):467–472
31. Zhu L, Chu H, Gu D, Ma L, Shi D, Zhong D, Tong N, Zhang Z, Wang M (2012) A functional polymorphism in miRNA-196a2 is associated with colorectal cancer risk in a Chinese population. DNA Cell Biol 31(3):350–354
32. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A (2004) Identification of mammalian microRNA host genes and transcription units. Genome Res 14(10a):1902–1910
33. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Getts R, Haqq C (2006) Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. Mol Cancer 5:24
34. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM (2005) MicroRNA gene expression deregulation in human breast cancer. Cancer Res 65:7065–7070