RESEARCH ARTICLE

Combined Genotype Analyses of Precursor miRNA-196a2 and -499a Variants with Hepatic and Renal Cancer Susceptibility- a Preliminary Study

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Abstract

MicroRNAs, a novel class of small non-coding RNAs, are key players in many cellular processes, including cell proliferation, differentiation, invasion and regeneration. Tissue and circulatory microRNAs could serve as useful clinical biomarkers and deregulated expression levels have been observed in various cancers. Gene variants may alter microRNA processing and maturation. Thus, we aimed to investigate the association of *MIR-196a2* rs11614913 (C/T), *MIR*-499a rs3746444 (A/G) polymorphisms and their combination with cancer susceptibility in an Egyptian population. Sixty five renal cell carcinoma (RCC) and 60 hepatocellular carcinoma (HCC) patients and 150 controls were enrolled in the study. They were genotyped using real-time polymerase chain reaction technology. Both *miR-196a2T and *miR-499a**G were associated with RCC risk, but only miR-196a*T was associated with HCC development. Carriage of the homozygote combinations (*MIR196a2**TT + *MIR499a**AA) and (*MIR196a2**CC + *MIR499a**GG) was associated with 25 and 48 fold elevation of likelhood to develop RCC, respectively. The *miR-196a2* SNP was also linked with larger tumor size in RCC and advanced tumor stage in HCC. miR-196a2 and miR-499a combined genotypes were associated with RCC and HCC. Further functional analysis of SNPs is required to confirm relationships between genotypes and phenotypes.**

Keywords: MIR-196a2 - MIR-499a - SNP - HCC - RCC

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Introduction

Cancer is characterized by unrestricted proliferation, invasion, and metastasis (Visone and Croce, 2009). Hepatocellular carcinoma (HCC) is the most common primary malignancy in liver, where it became the fifth common malignancy worldwide more so in Asia and Africa (Popat et al., 2013; Kar, 2014). Excessive liver cell turnover triggered by chronic liver injury and regeneration is the main cause of malignant transformation of hepatocytes. Such process can result in genetic alterations such as tumor suppressor gene inactivation, oncogene activation or genomic instability, including defects in DNA mismatch repair and chromosomal segregation (Moradpour and Blum, 2005). The main risk factor of HCC is liver cirrhosis that may occur due to different etiological factors such as viral (chronic hepatitis B and hepatitis C), toxic (alcohol and aflatoxins), metabolic (diabetes and non-alcoholic fatty liver disease, hereditary haemochromatosis) and immune-related (primary biliary cirrhosis and autoimmune hepatitis) (Kar, 2014). Renal cell carcinoma (RCC) on the other hand is the most common cancer of renal parenchyma in adults (Muglia and Prando, 2015). Major risk factors of RCC are diabetes, hypertension, obesity, cigarette smoking and family history of cancer (Chow et al., 2010). RCC originates from renal epithelium, accounts for 85% of renal cancers and about one fourth of the patients present with advanced stage of the disease with metastasis. In addition, RCC is characterized by the high recurrence rate (Cairns, 2010).

MicroRNAs (miRNAs) are small, single-stranded, noncoding, 19–21 nucleotide long RNA molecules. They act as negative regulators that involve post-transcriptional silencing of the gene expression through binding to target mRNAs regions and resulting in mRNA cleavage or repression (Valinezhad Orang et al., 2014). MiRNAs regulate the expression of about 10–30% of the all human genes through post-transcriptional mechanisms (Geeleher et al., 2012). Studies have revealed that some polymorphisms (SNPs) found in the miRNA genes

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can alter miRNA expression and/or maturation and be associated with the development and progression of cancer (Ryan et al., 2010). Two single nucleotide polymorphisms, miR-196a2 (rs11614913) and miR-499 (rs3746444) have been reported to be associated with cancer risk (Xu et al., 2011; He et al., 2012).

MiR-196a2 (rs11614913) is C to T variation located on chromosome 12. Studies showed that rs11614913 is associated with breast (Hu et al., 2009), Esophageal (Ye et al., 2008), colorectal (Min et al., 2012), non–small cell lung cancers (Hu et al., 2008) and hepatocellular carcinoma (Min et al., 2012), whereas MIR-499 (rs3746444) is A to G variation located on chromosome 20 was shown to be associated with HCC (Xiang et al., 2012), colorectal (Min et al., 2012) and breast cancers (Hu et al., 2009). As there are no previous studies, up to the researchers knowledge, on the combined effects of these variants in HCC and RCC patients among our population, this study for the first time will aim to determine the association between rs11614913 and rs3746444 polymorphisms and susceptibility to hepatocellular carcinoma and renal cell carcinoma in Egyptian patients, and to further assess its impact on the clinical outcome of these patients.

Materials and Methods

Study participants

A total of 275 subjects (65 patients with renal cell carcinoma, 60 patients with hepatocellular carcinoma, and 150 age- and sex-matched controls) were enrolled in the current study. Control group was recruited from blood bank donors with no history of cancer or any other chronic disorders. Archived formalin-fixed paraffin embedded (FFPE) renal tumor specimens, dating back up to 6 years, were obtained for miRNA SNP identification. They were collected from private Pathology Labs in Ismailia and Port-said, Egypt. Renal tumor specimens were evaluated by a single pathologist for determining the tumor type, size, pathological grade, and lymph node involvement. Hepatic cell carcinoma patients were retrieved from the inpatient ward of Tropical medicine and Gastroenterology Department, Assuit Hospitals. Blood samples were drawn from patients for genotyping analysis, during the period between July 2015 and December 2015. The staging was determined according to the TNM (tumor, node, metastasis) classification of the American Joint Committee on Cancer (AJCC, 7th edition). Thorough clinical examination was performed via a clinician. Laboratory and radiological investigations done previously to the hepatic patients were obtained from their records. The study was conducted in accordance with the guidelines in the Declaration of Helsinki and approved by the Medical Research Ethics Committee of Faculty of Medicine, Suez Canal University (approval no. 2774). Written informed consent was obtained from participants before taking part.

SNP identification

Genomic DNA from venous blood of normal controls and hepatic carcinoma patients was extracted using QIAamp DNA Blood Mini kit (Catalog No. 51104, QIAGEN, Germany) and from FFPE renal specimens using QIAamp DNA FFPE tissue kits (*Catalog No. 56404, QIAGEN, Germany*) according to the manufacturer's instructions. Extracted DNA concentration and purity were measured by NanoDrop ND-1000 (*NanoDrop Tech., Inc. Wilmington, DE, USA*). DNA samples of patients and controls were genotyped for the hsa-miR-196a2 and hsa-miR-499a polymorphisms. PCR was performed in a 25-μl reaction volume containing genomic DNA (20 ng) diluted to 11.25μL with DNase-RNase-free water, 12.5 μl Taqman® Universal PCR Master Mix; No AmpErase UNG (2x) and 1.25 μl 20 x TaqMan® SNP Genotyping Assay Mix (assay ID C_31185852_10 for rs11614913 and C_2142612_30 for rs3746444, *Applied Biosystems*). Genotyping was performed blinded to case/control status. Appropriate negative and positive controls were used. Real-Time PCR amplification was performed on StepOne™ Real-Time PCR System (*Applied Biosystems*) using the following conditions: two initial holds (50°C for 2 min and 95°C for 10 min) followed by a 40-cycle two-step PCR (95°C denaturation for 15 s and annealing/extension 60°C for 1 min). Allelic discrimination was called by the SDS software version 1.3.1 (*Applied Biosystems*). The overall genotype call rate by TaqMan allelic discrimination assays were 100% and the genotyping reproducibility was 100% in 25% of random samples.

Identifications of predicted target gene set

Multiple online computational tools were used to identify predicted target genes (in CDS, 3'UTR, or 5'UTR regions) for the studied miRNAs as miRTarBase v 20 (http://mirtarbase.mbc.nctu.edu.tw/), miRDB (http://mirdb.org), DIANA-microT-CDS v5.0 (http://www.microrna.gr/microT-CDS), PicTar (http://pictar.mdc-berlin.de/), and TargetScanHuman v6.2 (http://www.targetscan.org/).

The predicted miRNA target genes were analyzed for functional annotation clustering and Kyoto encyclopedia of genes and genomes (KEGG) enrichment pathways (Kanehisa et al., 2004) using DIANA-miRPath v2.0 web-server with default settings (Vlachos et al., 2012). In addition, the physical interaction relationships and biological functions of both miR-196a2 and miR-499 with genes of renal and hepatic cancer KEGG pathways were analyzed using miRTar.Human tool (http://miRTar.mbc.nctu.edu.tw/).

Predicted functional effect of rs11614913 and rs3746444 polymorphisms

The predicted functional impact of miR-196a* (5'-CGGCAACAAGAAACUGC[C/U]UGAG-3') and miR-499a* (5'AAC[A/G]UCACAGCAAGUCUGUGCU-3') polymorphisms at the 3p arm was performed using miRmut2Go (http://compbio.uthsc.edu/miR2GO), a web-based platform for comparative functional analysis of mutations in microRNAs based on the enriched functional annotations of reference and derived target gene sets (Bhattacharya and Cui, 2015). Selected parameter settings: union combine results using both TargetScan and miRanda methods for miRNA target prediction, the p value threshold for functional enrichment

set as <0.01, and moderate Gene Ontology hierarchical filtering level.

Statistical analyses

All the statistical analyses were performed using the "Statistical Package for Social Sciences (SPSS) for windows" software, version 20. Two-sided Ci squatre test was used. The allele frequency within each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. Hardy-Weinberg equilibrium (HWE) for each SNP was tested by using a goodness-of-fit χ2-test with df = 1 via the Online Encyclopedia for Genetic Epidemiology (OEGE) software (http://www.oege.org/software/hwe-mr-calc.shtml). The associations of rs11614913 and rs3746444 genotypes and susceptibility to each cancer type were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CI) from unconditional logistic regression analysis with the adjustment for possible confounders. P<0.05 was considered statistically significant. The statistical power was estimated using Quanto software version 1.2.4; (http://biostats.usc.edu/software, University of Southern California).

Results

Association of pre-miRNA variants with cancer risk and prognosis

Demographic characteristics of the study groups are shown in Supplementary Figure 1. The study included 125 patients (65 RCC and 60 HCC) and 150 healthy controls. Genotype distributions, allele frequencies and the carriage rate of the rs11614913 and rs3746444 SNP in the study population are shown in Tables 1 and 2, and Figure 1. The observed genotype frequencies in both cases and controls were in agreement with those expected by the HWE (all p values > 0.05). Minor allele frequencies (MAF) were 0.29 for rs11614913*T and 0.40 for rs3746444*G in controls. These alleles were significantly increased among RCC patients, accounting for 0.41 and 0.78 for miR-196a2*T and miR-499a*G (p=0016 and <0.001, respectively). However, no significant difference was observed in the allele distribution in HCC patients compared to controls; 0.32 for MAF of miR-196a2*T (p=0.589) and 0.34 for miR-499a*G (p=0.267) in patients.

Single SNP analysis of RCC patients revealed significant association of both miR-196a2 and miR-499a genotypes and alleles with disease risk, Table 1. Genetic association models showed that individuals with miR-196a2*T variant were more likely to develop cancer under heterozygote, dominant and allelic models (CT versus CC: OR = 2.03, 95% CI = 1.07-3.86, TT+CT versus CC: OR = 2.08, 95% CI = 1.14-3.8, and T versus C: OR = 1.69, 95% CI = 1.09-2.58). On the other hand, miR-499a*G allele showed twice more susceptibility to RCC than A carriers under dominant, recessive, and allelic genetic models (AG+GG versus AA: OR = 6.02, 95% CI = 24-148, GG versus AA+AG: OR = 8.3, 95% CI = 4.3-16, and

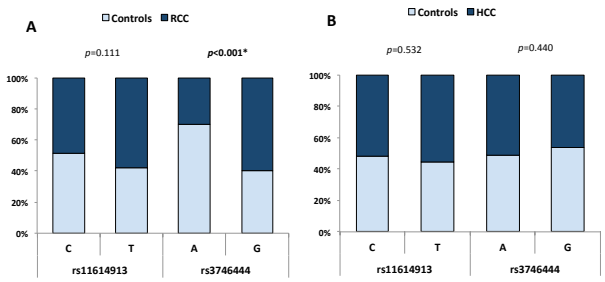

Figure 1. Carriage Rates of rs11614913 and rs3746444 Alleles in the Study Population. RCC, renal cell carcinoma; HCC, hepatic cell carcinoma. Chi square test was used. *Statistically significant at p<0.05

Table 1. Genotype and Allele Frequencies of *hsa-miR-196a2* (rs11614913: C>T) and *hsa-miR-499a* (rs3746444: A>G) Polymorphisms in Renal Cell Carcinoma Patients and Controls

Genetic model	Genotype	Controls (n=150)		RCC (n=65)		P value	OR (95% CI)	
MIR-196a2 rs11614913								
Co-dominant model a	CC	80	(53.3)	23	(35.4)	0.024* [b]		Reference
	CT	53.0	(35.3)	31	(47.7)		2.03	(1.07-3.86)
	TT	17.0	(11.4)	11	(16.9)		2.25	(0.92-5.47)
P-HWE		0.082		0.919				
Dominant model	CC	80	(53.3)	23	(35.4)	0.015*		Reference
	CT + TT	70	(46.7)	42	(64.6)		2.08	(1.14-3.80)
Recessive model	CC+CT	133	(88.7)	54	(83.1)	0.264		Reference
	TT	17	(11.3)	11	(16.9)		1.59	(0.70-3.62)
Allelic model	C	213	(71.0)	77	(59.2)	0.016*		Reference
	T	87	(29.0)	53	(40.8)		1.69	(1.09-2.58)
MIR-499a rs3746444								
Co-dominant model b	AA	57	(38.0)	6	(9.2)	<0.001*		Reference
	AG	66	(44.0)	17	(26.2)		2.44	(0.90-6.62)
	GG	27	(18.0)	42	(64.6)		14.7	(5.6-38.9)
P-HWE		0.307		0.047				
Dominant model	AA	57	(38.0)	6	(9.2)	<0.001*		Reference
	AG+GG	93	(62.0)	59	(90.8)		6.02	(2.4-14.8)
Recessive model	AA+AG	123	(82.0)	23	(35.4)	<0.001*		Reference
	GG	27	(18.0)	42	(64.6)		8.3	(4.3-16)
Allelic model	A	180	(60.0)	29	(22.3)	<0.001*		Reference
	G	120	(40.0)	101	(77.7)		2.3	(1.4-3.7)

Values are shown as number (%). HWE P; p value of Hardy-Weinberg equilibrium. OR (95% CI), odds ratio and confidence interval. (a) Represented both heterozygote and homozygote comparison models. Chi square (c2) test were used. (b) P values by Chi-square for trend. * Statistically significant at p<0.05

Table 2. Genotype and Allele Frequencies of *hsa-miR-196a2* (rs11614913: C>T) and *hsa-miR-499a* (rs3746444: A>G) Polymorphisms in Hepatocellular Carcinoma Patients and Controls

Genetic model	Genotype	Controls (n=150)		HCC (n=60)		P value	OR (95% CI)	
		MIR-196a2 rs11614913						
Co-dominant model[a]	CC	80	(53.3)	25	(41.7)	0.041*[b]		Reference
	CT	53	(35.3)	32	(53.3)		1.9	(1.03-3.6)
	TT	17	(11.4)	3	(5.0)		0.56	(0.15-2.08)
P-HWE		0.082		0.071				
Dominant model	CC	80	(53.3)	25	(41.7)	0.126		Reference
	CT + TT	70	(46.7)	35	(58.3)		1.6	(0.8-2.9)
Recessive model	CC+CT	133	(88.7)	57	(95.0)	0.157		Reference
	TT	17	(11.3)	3	(5.0)		0.4	(0.11-1.46)
Allelic model	C	213	(71.0)	82	(68.3)	0.589		Reference
	T	87	(29.0)	38	(31.7)		1.13	(0.71-1.79)
		MIR-499a rs3746444						
Co-dominant model[b]	AA	57	(38.0)	28	(46.7)	0.51		Reference
	AG	66	(44.0)	23	(38.3)		0.7	(0.3-1.3)
	GG	27	(18.0)	9	(15.0)		0.6	(0.28-1.6)
P-HWE		0.307		0.251				
Dominant model	AA	57	(38.0)	28	(46.7)	0.248		Reference
	AG+GG	93	(62.0)	32	(53.3)		0.7	(0.38-1.28)
Recessive model	AA+AG	123	(82.0)	51	(85.0)	0.602		Reference
	GG	27	(18.0)	9	(15.0)		0.8	(0.35-1.82)
Allelic model	A	180	(60.0)	79	(65.8)	0.267		Reference
	G	120	(40.0)	41	(34.2)		0.7	(0.5-1.2)

Values are shown as number (%). HWE P; p value of Hardy-Weinberg equilibrium. OR (95% CI), odds ratio and confidence interval. (a) Represented both heterozygote and homozygote comparison models. (b) Chi square (c2) or Fisher's exact tests were used. * Statistically significant at p<0.05.

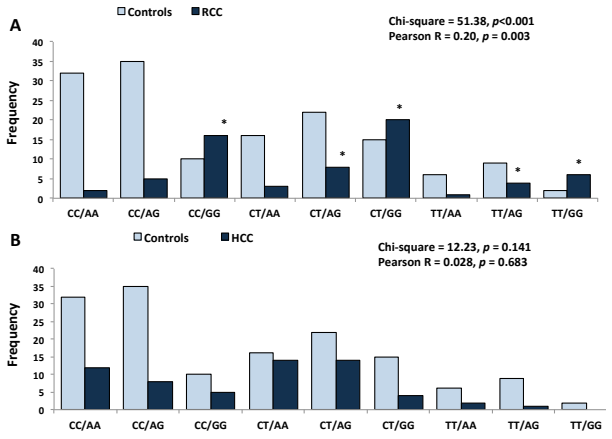

Figure 2. Association of *hsa-miR-196a2* (C/T) and *hsa-miR-499a* (A/G) Genotype Combinations with (A) Renal and (B) Hepatic Cancer Risk. (*) statistically significant compared to the wild type (CC/AA)

G versus A: OR = 2.3, 95% CI = 1.4-3.7). Stratification analysis by gender revealed a positive association of rs11614913*T with RCC risk in male patients (p=0.014) and rs3746444*G variant with RCC in both male and female patients (p<0.001), Supplementary Figure 2. Genotype combination analysis of the two studied SNPs showed that individuals with the homozygote forms TT/GG and CC/GG had the highest risk of RCC with OR (95% CI) of 48 (5.6-410) and 25.6 (5-131), respectively. Following this, the combination of heterozygote genotype CT or AG with the risk allele previously identified by the single SNP analysis were more likely to have renal cancer compared to the wild type CC/AA genotypes [CT/GG: 21.3 (4.4-103.3), TT/AG: 7.1 (1.11-45.2), CT/AG: 5.8 (1.12-30.04)] (Figure 2).

With regards to HCC, there was genotype differences of MIR-196a2 SNP among patients and controls (p=0.041). Heterozygote genotype was more prevalent in HCC patients (53.3%) compared to healthy controls (35.3%), with OR (95% CI) of 1.9 (1.03-3.6). No differential effect was observed on different genotype combinations of miR-196a2 and miR-499a (Figure 2). Stratified analysis by gender illustrated no gender-specific associations of the two SNPs with HCC.

The associations of rs11614913 and rs3746444 polymorphisms with the clinicopathological characteristics of cancer patients was also investigated. Association analysis showed that the heterozygote rs11614913*CT genotype was significantly associated with advanced tumor size (p = 0. 020) in RCC cases, while carriers of homozygote rs11614913*TT genotype had significantly more advanced tumor TNM stage (p=0.047) in HCC patients. In contrast, the rs3746444 SNP did not show significant association with any clinicopathological characteristics.

In silico data analysis

Human MIR-196a2 and miR-499a genes are 121 and 122 base pairs in length, respectively. During the biogenesis process, precursor hairpin transcripts undergo two cleavage processes. Each stem loop produces two different mature forms from 5p and 3p arms. Hundreds of genes were predicted to be targeted by miR-196a2 and miR-499a. Enriched KEGG pathway analyses using miRTar.Human tool (http://miRTar.mbc.nctu.edu.tw/) and DIANA-miRPath v2.0 web-server (at threshold 0.7) showed that the targeted genes regulated by mir-196a2 and miR-499a were involved in essential biological processes in cancer pathways, and in particular renal cell carcinoma [KEGG hsa05211], Figure 3.

The variants of both miR-196a2 and miR-499 are located in the passenger strand at the 3' and 5' ends, respectively. *In silico* analysis revealed no functional effect of rs11614913 polymorphism on the predicted target gene sets. While the rs3746444 SNP of the miR-499a is existed inside the 'seed region' responsible for miRNA complementation with mRNA targets, thus each allele has its own targets (PolymiRTS Database 3.0).

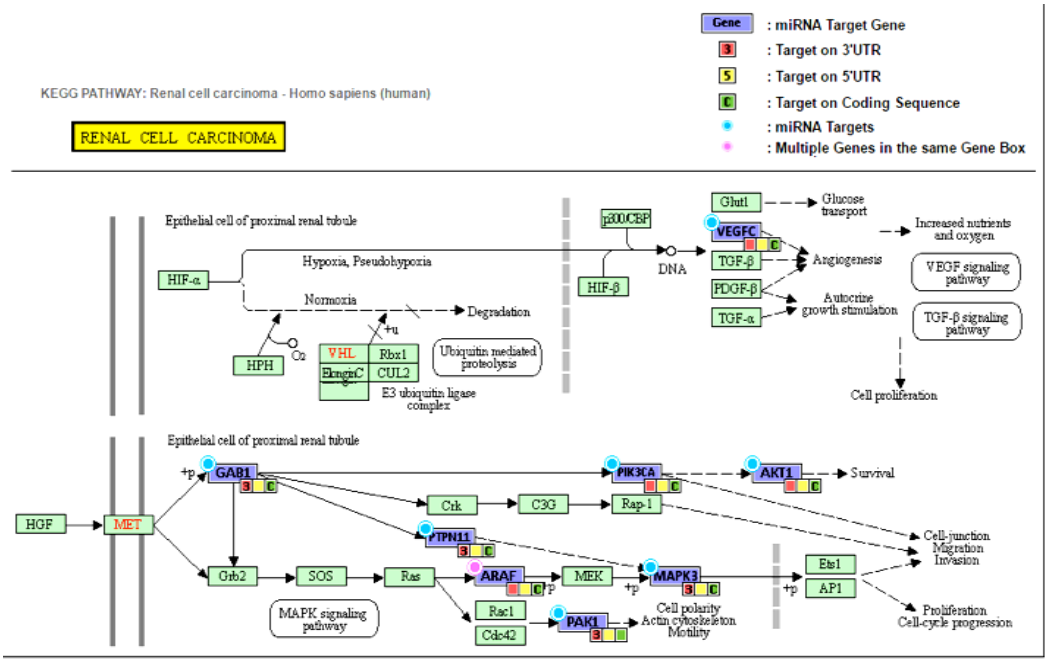

Figure 3. Predicted Target Genes of *miR-196a2* and *miR-499a* in Renal Cell Carcinoma Pathway [KEGG: hsa05211]

Discussion

Single nucleotide polymorphisms in miRNA and their targets have been associated with an increased risk of many types of cancer including HCC and RCC. Because miRNAs must closely recognize binding sites in their target genes, a variation in even one nucleotide may produce dramatic changes in the post-transcriptional regulation of their target genes (Morishita and Masaki, 2015). The impact of a given SNP will depend on its functional effects on the miRNA itself and the degree to which the native miRNA affects the expression of cancer driving genes. The selected SNPs in the current study, may affect the miRNA expression and its potential targets, and therefore, might play a role in the regulatory processes during cancer development (Ryan et al., 2010).

Many miRNA variants have been associated with HCC risk as miR-101-1; rs7536540 and miR-101-2; rs12375841 (Bae et al., 2012), miR-34b/c; rs4938723 (Xu et al., 2011) and miR-106b-25-cluster; rs999885 (Liu et al., 2012). All were positively associated with an increased risk of HCC. In contrast, miR-371–373; rs3859501 (Kwak et al., 2012) and miR-149c; rs2292832 (Kim et al., 2012) were negatively involved in HCC risk. However, conflicting results were obtained from several studies regarding miR-196a2; rs11614913 (Li et al., 2010; Akkiz et al., 2011a; Guo et al., 2012; Kim et al., 2012) and miR-499a; rs3746444 (Akkiz et al., 2011b; Kim et al., 2012; Xiang et al., 2012). As these latter miRNAs are important in the carcinogenic process, we hypothesized that their precursors rs11614913 (C/T) and rs3746444 (A/G) SNPs are associated with cancer risk.

We found that individuals who carried the rs11614913 CT/TT genotypes had an overall increased risk of HCC and RCC cancers compared with the CC genotype. This was consistent with previous studies in which it has been found to be associated with the susceptibility to hepatitis B virus (HBV) -related HCC in male Chinese patients (Li et al., 2010; Qi et al., 2010) and in the Turkish population (Akkiz et al., 2011a), whereas contradictory to others (Kim et al., 2012). In line with our findings, Hou et al., (2010), suggested that miR-196a2 could play an important role in HCV-related HCC through the inhibition of Bach1 (a basic leucine zipper mammalian transcriptional repressor) and upregulation of hemeoxygenase 1. Regarding RCC, Du et al. (2014), found that the MIR-196a2 SNP was associated with RCC susceptibility in a recessive model and the rs11614913 CC genotype was associated with a significantly decreased expression of miR-196a-5p in their 26 renal cancer tissues. Moreover, their luciferase reporter assays revealed the potential effect of rs11614913 SNP on the binding of miR-196a-3p to its targets. Since the target genes for miR-196a include mediators of apoptosis and Hox genes, its aberrant expression can lead to severe changes in cellular pathways and initiate the process of tumorigenesis (Hornstein et al., 2005). In addition, a previous functional work by Hoffman et al., (2009), showed that the T allele altered the expression of less than half of the number of transcripts altered by the C allele miRNA in vitro, supporting that the change in sequence in the T allele may result in a diminished capacity to regulate its targets.

On the other hand, the MIR-499a A>G rs3746444 polymorphism has no significant role in the genetic susceptibility to hepatocellular carcinogenesis in our study. In line with this observation, Chen et al., (2014), meta-analysis results indicated increased risks were found in Asians and Iranians, but not in Caucasians in all genetic models tested. However, there were conflicting data according to different populations [Akkiz et al., 2011b; Kim et al., 2012; Xiang et al., 2012). Frequencies of specific genotypes are variable in different ethnic groups

and this could partly explain the difference in risk among different populations. In addition to differences between the studies in sources of controls, disease stages and/or sample sizes.

Our *in silico* analysis predicted hundreds of target genes for miR-196a2 and miR-499. They were involved in multiple functional pathways, including TGFβ (transforming growth factor beta) signaling, VEGF (vascular endothelial growth factor) signaling pathway, immune cell trafficking, focal adhesion, cell-to-cell signaling, tight junction, mTOR (mammalian target of rapamycin) signaling, apoptosis, tissue remodeling, invasion and metastasis. Specifically, in pathways in cancer (hsa05206) and RCC pathway (hsa05211), miR-196a2 can target MAPK3 (mitogen activated protein kinase 3), ARAF (proto-oncogene belongs to the RAF subfamily of the Ser/Thr protein kinase family), and PTPN11 (phosphatase non-receptor type 11) involved in tissue invasion, cell migration and proliferation. In addition, CDK2 (cyclin-dependent kinase 2), PIK3CA (Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase, Catalytic Subunit alpha), WNT5A (wingless-type MMTV Integration site family, member 5A), HSP90 (90 kDa heat shock protein) and AKT1 (Protein Kinase B) genes are essential for survival and cell proliferation. Whereas, miR-499a target ARNT2 (aryl hydrocarbon receptor nuclear translocator 2) and ETS1 (E26 transformation-specific transcriptional factor 1) via VEGF signaling pathway leading to sustained angiogenesis. It is also implicated in genes involved in tissue invasion and metastasis via β-catenin pathway as CTNNA1 [Catenin (Cadherin-Associated Protein), Alpha 1], and PRKACA (protein kinase, CAMP-activated catalytic, subunit alpha). Moreover, MDM2 (mouse double minute 2 homolog proto-oncogen) and TRAF5 (Tumor necrosis factor receptor-associated factor 5) gene targets are known to regulate apoptosis.

Previous researches reported that SOX6 and Rod1 genes are two direct targets of miR-499. The SOX6 induces G1/S cell cycle arrest by up-regulating p53 and p21, and down-regulating cyclin D1/CDK4, cyclin A, and β-catenin (Kim et al., 2012). SOX6 can also suppress the development of esophageal squamous cell cancer (Qin et al., 2011). Rod1 represses cell migration and induces apoptosis, reducing the risk of gastric cancer (Tano et al., 2010).

Since the expression of miRNAs is highly tissue specific, changes within the miRNA sequence can indeed specifically predispose to cancers of particular organs and mediate different molecular changes in different tissues (De la Chapelle and Jazdzewski, 2011). This could in part explain the cancer risk our studied SNPs implied in one type of cancer in our cases and absence of this risk in another type.

We confirm that to study the relationship between any microRNA and cancer, it is important to examine not only the gene variants but also the aberrant expressions of these microRNA and their target genes; this is highly recommended in future work. Additional large studies with ethnically diverse populations are warranted to further confirm the impact of the studied miRNA polymorphisms on cancer susceptibility and to explore their potential therapeutic effects, such as improvement in sensitivity to radio- and chemotherapy.

In conclusion, in this pilot study, we observed some evidence of an association between MIR-196a2 rs11614913 polymorphism with the risk of HCC and RCC cancers and lack of such association regarding MIR-499a with HCC in our population.

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