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Original article

The correlation between miR-146a C/G polymorphism and *UHRF1* gene expression level in gastric tumor

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OBJECTIVE: To investigate the association between the polymorphism of miR-146a and The ubiquitin-like with PHD and ring-finger domains 1 (*UHRF1*) expression in patients with gastric cancer.

METHODS: MiR-146a rs2910164 was genotyped in 130 patients with gastric cancer and 130 cancer-free individuals using polymerase chain reaction (PCR)-restriction fragment length polymorphism. *UHRF1* expression was analyzed in 22 gastric cancer tissues and their adjacent normal tissues using quantitative real-time PCR.

RESULTS: No significant differences in genotype distributions of miR-146a rs2910164 were found between cases and controls, but we observed that grade II tumors were more frequently detected in patients with CG/CC genotype compared to those with CC genotype. *UHRF1* expressions in cancerous tissues were significantly higher than in noncancerous tissues (1.89-fold). Patients with CC genotype showed a significant increase in *UHRF1* expression in comparison to the carriers of GG/CG genotype. A higher *UHRF1* expression was associated with cancer stage IV and grade III ($P<0.05$).

CONCLUSION: The overexpression of *UHRF1* was correlated with the stage and grade of gastric cancer and is associated with the genotype distribution of rs2910164.

KEY WORDS: miR-146a, microRNAs, single nucleotide polymorphism, stomach neoplasms, UHRF1.

INTRODUCTION

Gastric cancer is one of the most common gastrointestinal cancers and is recognized as the third leading cause of cancer-related death worldwide in 2012.[1,2] In fact, gastric cancer is a complex disease which may result from an interaction between genetic and environmental factors.[2] So far, the molecular mechanisms involved in gastric carcinogenesis have not been fully understood.

The ubiquitin-like with PHD and ring-finger domains 1 (UHRF1) belongs to the UHRF family and, as an epigenetic factor, it plays a pivotal role in the carcinogenesis by participating in epigenetic mechanisms including DNA methylation, histone deacetylation, histone methylation and, probably, histone ubiquitination.[3–7] In non-cancerous cells, cell cycle progression requires *UHRF1* expression for G1/S transition.[8] As an oncogene, *UHRF1* has been proven to be overexpressed in various types of cancers

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including breast, prostate, lung, bladder, colorectal cancers and, recently, in gastric cancer.[9–14] Although the biological activities of *UHRF* gene are not accurately known, much evidence shows the fundamental role of *UHRF* gene in epigenetic, cell proliferation and apoptosis processes.[15] *UHRF1* overexpression leads to the migration and invasion of gastric cancer cells.[14] Among different regulatory mechanisms of gene expressions, microRNAs (miRNAs) act as a negative regulator at the posttranscriptional level of gene expression.[16] miRNA is the non-coding small RNA of 21–24 nucleotides, which regulates gene expression by imperfect pairing with the 3′-untranslated region (3′ UTR) of the target mRNA.[16] Single nucleotide polymorphisms (SNPs) of miRNA have attracted considerable attentions in recent years. Extensive studies have proven that the SNPs in miRNAs and their target sites may be involved in posttranscriptional regulation of gene expression.[16–21]

Previous epidemiological studies have indicated that the presence of the G>C genetic variant (rs2910164), which is located within the seed sequence of the miR-146a precursor (pre-miR-146a), can change the susceptibility to different types of cancers and diseases.[19–29]

MiR-146a has 224 potential mRNA-binding targets, including cancer susceptibility gene *UHRF1*.[14,30] Zhou *et al.* suggested that miR-146a, as a tumor suppressor, could decrease *UHRF1* expression by binding its 3′UTR sequence.[14] A correlation analysis showed that *UHRF1* overexpression and miR-146a downregulation were correlated with the progression of gastric cancer.[14] A similar study showed the control effect of miR-146a on *RNASEL* gene in the SNP–SNP interaction of these genes among patients with non-melanoma skin cancer.[30] Moreover, G/C SNP of pre-miR-146a is associated with reduced miR-146a levels in the GG genotype compared with those in the CC genotype in gastric cancer.[31]

The identification of a new regulatory relationship between miR-146a and *UHRF1* gene expression shows that there is a close relationship between genetic and epigenetic factors in the development and progression of gastric cancer and any variation in the miR-146a sequence may downregulate the expression of *UHRF1*. This study aimed to assess the relationship between *UHRF1* expressions and pre-miR-146a genotypes in Iranian gastric cancer patients of Lor ethnicity.

MATERIALS AND METHODS

Clinical samples and genotyping of miR-146a rs2910164

Blood samples were collected from 130 patients with gastric cancer and 130 age-matched and sex-matched cancer-free controls to investigate the genotypes of miR-146a polymorphism. In addition, the tumor tissues were obtained from 22 patients during endoscopy or surgery. All samples were collected at the Lorestan University of Medical Sciences (Khorramabad, Iran) between October 2013 and July 2015. Both groups were of Lor ethnicity. The patients' pathological results (including tumor grade) were also collected for further analysis. This study was approved by a local Ethical Committee based on the rules and regulations of the Iranian Ministry of Health, and all participants provided their written informed consents.

DNA extraction from blood samples as well as gastric cancer tissues was carried out for SNP genotyping by using a DNG-plus solution kit (SinaClon BioScience Co., Tehran, Iran). Polymerase chain reaction (PCR) amplifications were performed with forward (5′-TGCTGTGACAGGCAGAGCAG-3′) and reverse primers (5′-GCCTTAGGTGACTGGAGGCCTG-3′). Each PCR reaction was carried out in a total volume of 20 μL containing 150 ng of genomic DNA, 10 pmol of each primer and, 10 μL of 2X Tag Premix (Parstous, Mashhad, Iran).

PCR amplification cycle conditions involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles with three steps: at 94 °C for 20 s, at 68 °C for 20 s and at 72 °C for 45 s. This was followed by a final extension at 72 °C for 5 min. The PCR products were digested with *Sac*I (1 μL) at 37 °C for 30 min. The digested products were visualized by 3% agarose gel electrophoresis followed by DNA stain with a green viewer. Some of the PCR products were sequenced for genotype confirming.

UHRF1 gene expression

Altogether 22 gastric cancer tissues and their adjacent normal tissues were collected, immediately snap frozen in liquid nitrogen and stored at −80 °C for further analysis. Total RNA of the tissue samples was extracted using the Trizol RNA isolation reagent (SinaClon BioScience Co.) according to the manufacture's protocol. The extracted RNA was treated by DNase to remove DNA contamination. The quantity and quality of RNA were measured using the NanoDrop

2000 Spectrophotometer (Thermo Fisher Scentific, Waltham, MA, USA) and 0.8% agarose gel electrophoresis, respectively. The cDNA of each sample was synthesized by a PrimeScript RT reagent kit (TaKaRa, Tokyo, Japan) according to the manufacturer's instructions.

The *UHRF1* gene expression was evaluated by using SYBR Green Premix with human glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a reference gene. The qPCR was performed in the Light Cycler 6000 detector (Qiagen, Hilden, Germany). The PCR reaction was prepared in a total volume of 20 μL containing 10 μl of SYBR Green Premix (Parstous), 1 pmol of each forward and reverse primer, and 2 μL cDNA. The primer for *UHRF1* and *GAPDH* genes were as follows: *UHRF1*, forward 5′-CCAGCAGAGCAGCCTCATC-3′, reverse 5′-TCCTTGAGTGACGCCAGGA-3′; and *GAPDH*, forward 5′-TGATGACATCAAGAAGGTGGTGAAG-3′, reverse 5′-TCCTTGGAGGCCATGTGGGCCAT-3′. Amplification program was performed at 95 °C for 30 s as an initial denaturation step, followed by 45 cycles at 95 °C for 3 s and at 58 °C for 30 s.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) of the pre-miR-146a genotypes was examined using the χ^2 test. Odds ratio (OR) and 95% confidence interval (CI) were calculated to analyze the relationship between the miR-146a polymorphism and the risk of gastric cancer. The most frequent homozygotes were used as a reference. All genotype analyses were performed using SPSS 21 (IBM, Armonk, NY, USA). Relative expression was calculated using the ΔΔCt model (ΔCt *UHRF1*- ΔCt GAPDH) with REST Software 2.0.13 (Qiagen). *P* value ≤ 0.05 was considered statistically significant.

RESULTS

Characteristics of the participants

The mean age of patients and cancer-free controls was 67 ± 14 years and 61 ± 13 years, respectively. Based on the pathological results, the frequencies of tumor grades I, II and III were 8.5%, 16.1% and 75.4%, respectively. Of the participants 59.2% had an urban lifestyle and 40.8% had a rural one.

Genotype distributions of miR-146a polymorphism and the risk of gastric cancer

The HWE showed no significant differences between the genotype frequencies in cases and controls (*P* = 0.06 and *P* = 0.10, respectively). Although no significant relationship was found in the overall statistical analysis between pre-miR-146a gene genotypes and the risk of gastric cancer (Tables 1 and 2), grade II tumors were more frequently detected in patients with a CG/CC genotype compared with those with a GG genotype (Table 3).

Expression of *UHRF1* gene in gastric cancer patients and pathological associations

Our study showed that the expression of *UHRF1* gene was upregulated in cancer tissues than in the adjacent

Table 1. miR-146a rs2910164 G/C genotype and allele frequency distribution in gastric cancer patients and controls

Genotype	Cases *n* (%)	Controls *n* (%)	OR (95% CI)	*P* value
CC	13 (10.0)	10 (7.7)	1.3 (0.5–3.35)	0.40
CG	42 (32.3)	40 (30.8)	1.12 (0.6–1.91)	0.60
GG	75 (57.7)	80 (61.5)	1	
CG + CC	55 (42.3)	50 (38.5)	1.17 (0.7–1.9)	0.50
G-allele	192 (73.8)	200 (76.9)	1	
C-allele	68 (26.2)	60 (23.1)	1.18 (0.7–1.7)	0.40

CI, confidence interval; OR, odds ratio.

Table 2. Stratified analyses for variant pre-miR-146a C/G genotypes in cases and controls

Variable	(CG + CC)/GG		OR (95% CI)	*P* value
	Cases	Controls		
Sex				
Female	18/22	15/21	1.14 (0.46–2.84)	0.70
Male	37/53	35/59	1.17 (0.6–2.12)	0.50
Age				
≤ 50 years	6/17	16/22	0.4 (0.1–1.5)	0.20
> 50 years	49/58	34/58	1.4 (0.8–2.5)	0.20

CI, confidence interval; OR, odds ratio.

Table 3. Correlation of the polymorphism of pre-miR-146a with tumor grade and place of residence

Variable	CG + CC	GG	OR (95% CI)	*P* value
Tumor grade				
G3	37	61	1	
G2	15	6	4.12 (1.4–11.55)	0.0047
G1	3	8	0.6 (0.1–2.47)	0.50
Residence				
Urban	38	39	1	
Rural	17	36	0.4 (0.2–1.0)	0.07

CI, confidence interval; OR, odds ratio.

normal tissues. Moreover, *UHRF1* gene expression was significantly upregulated in female patients compared with male patients. Its expression was also upregulated in tumors at stage IV and grade III by almost threefold compared with that at an early stage and grade (Table 4). However, no significant increase in *UHRF1* gene expression was observed at early stages and grades.

The influence of the pre-miR-146a G/C polymorphism on *UHRF1* gene expression

UHRF1 gene expression and rs2910164 genotypes were determined in 22 untreated gastric cancer tissue samples. The GG, CG and CC genotype distributions were found in 13 (59.1%), 5 (22.7%), and 4 (18.2%) patients, respectively. The correlation analysis showed that patients with the CC genotype had a 6.892-fold increase in *UHRF1* expression compared with those with GG/CG genotypes ($P = 0.001$, Table 4).

DISCUSSION

The geographical distribution of the incidence of gastric cancer has changed considerably over the past few decades. The incidence has decreased in the United States but increased in Asian countries, especially in Japan and Korea.[32] In spite of some known nutritional risk factors, genetic defects are significantly related to the susceptibility to gastrointestinal cancers.[33] Changes in *UHRF1* expressions appear to be related to the stages of cancer. The intensity of its overexpression could be a useful diagnostic marker for anti-cancer treatment.[34,35] Several studies have shown changes in nucleotide pairs (G:U to C:U) in the stem structure of pre-miR-146a. This is related to the alteration of miR-146a expression and its regulatory effect on the target genes in various types of human cancers and diseases.[19,21,36,37]

Our study aimed first, to analyze the relationship between the expression of *UHRF1* gene and the different stages of gastric cancer and second, to investigate the relationship between the SNP C/G genotype of miR-146a and changes in *UHRF1* gene expression in patients with gastric cancer.

Although no significant association was observed between the genotypes and alleles of the miR-146a polymorphism and the risk of gastric cancer, we found that grade II tumors were more frequently detected in patients with CG/CC genotype compared with those with GG genotype. Similar to our findings, some reports on hepatocellular carcinoma, breast cancer, non-small cell lung cancer, squamous cell carcinoma of the head and neck and bladder cancer showed no relationship between miR-146a polymorphism and cancer risk.[22–26,38] In contrast, many studies have reported that the risk of cancer was correlated with the genotype of pre-miR-146a in various cancers such as prostate cancer, esophageal squamous cell carcinoma, familial breast/ovarian cancer and gastric cancer.[20,27–29] Based on these evidence, it has been suggested that the differences in the results of various studies may be due to ethnic variations and the effect of various environmental factors. Our study showed that the upregulation of *UHRF1* gene expression was significantly associated with cancer stage IV and grade III.

Table 4. Correlation of *UHRF1* gene expression with sex, clinicopathological features and pre-miR-146a polymorphism genotypes

Variable	Type	Reaction efficiency	Expression	SE	95% CI	P(H1)	Result
GAPDH	REF	0.8449	1.000				
Total patients	TRG	0.8772	1.893	0.519–8.177	0.032–56.669	0.011	UP
Female	TRG	0.8607	3.403	0.916–20.066	0.016–77.893	0.044	UP
Male	TRG	0.8698	1.471	0.524–4.193	0.025–63.667	0.233	
Stage I, grade I	TRG	0.8478	1.242	0.314–4.852	0.048–14.274	0.547	
Stage II, grade II	TRG	0.8538	2.093	0.716–6.604	0.262–14.629	0.014	UP
Stage IV, grade III	TRG	0.815	3.426	1.264–9.535	0.742–26.051	0.001	UP
Individuals with GG genotype	TRG	0.861	2.423	0.679–12.860	0.026–92.049	0.020	UP
Individuals with CC	TRG	0.8819	6.892	1.619–45.872	0.958–143.135	0.001	UP
Individuals with CG	TRG	0.9085	0.726	0.117–4.366	0.019–11.467	0.564	

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; P(H1), probability of alternate hypothesis that difference between sample and control groups is due only to chance; REF, reference gene; TRG, target gene; UP, upregulated.

The variation of *UHRF1* expression is significant at different stages of cancer. It should be noted that in the present study, the changes in the gene expression of *UHRF1* based on qPCR analysis are in agreement with previous studies that evaluated the correlations between *UHRF1* mRNA and their proteins with cancer risk.[11–14,39,40] Consistent with these findings, Unoki *et al.* demonstrated that *UHRF1* was overexpressed in the grades II and III bladder tumors than in normal tissues, benign kidney tumors and several other kinds of normal tissues.[12] Furthermore, an increased expression of *UHRF1* in colorectal cancer tended to be associated with the depth of invasion in the malignant transformation of colon cancer, supporting the role of *UHRF1* in carcinogenesis.[13] Studies on lung cancer and cervical neoplasia have also confirmed that changes in *UHRF1* expression at different cancer stages can be used as a marker in discriminating between high and low grades of cancer.[11,34] The *UHRF1* expression was observed in all histological types of lung cancer, especially in non-adenocarcinomas. The high expression of *UHRF1* was associated with poor prognosis. Although *UHRF1* overexpression was associated with malignant indicators, *UHRF1* was detectable in half of the lung cancer patients at an early pathological stage. Overall, *UHRF1* is upregulated in various cancers and in the present study *UHRF1* overexpression confirmed its role in carcinogenesis of gastric cancer. A comparison of changes in the expression of *UHRF1* and rs2910164 genotype distribution showed that *UHRF1* mRNA expressions in CC genotype patients was much higher than in patients carrying GG/CG genotypes.

In our study, eight of the ten samples that showed no variation in *UHRF1* gene expression had the GG genotype, indicating that the G allele may play a protective role against the overexpression of *UHRF1* in patients with GG genotype. The positive correlation between increased *UHRF1* expression and the genotypes of miR-146a, especially in women, reveals the specific effect of the miR-146a genotype on sex; however, further studies are needed to investigate this sex-specific association.

In conclusion, the upregulated gene gene expression of *UHRF1* was significantly associated with higher grades of tumor, female gender, CC and GG genotypes. Therefore, *UHRF1* overexpression may be affected by SNP rs2910164 variation and probably influences the outcomes of gastric cancer. Further studies are needed to confirm the relationship of *UHRF1* gene expression and miR-146a polymorphisms in gastric cancer metastasis.

REFERENCES

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69–90.
2 Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. *Gastroenterology* 2012; **143**: 550−63.
3 Bostick M, Kim JK, Estève P-O, Clark A, Pradhan S, Jacobsen SE. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* 2007; **317**: 1760−4.
4 Sharif J, Muto M, Takebayashi S *et al.* The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 2007; **450**: 908–12.
5 Unoki M, Nishidate T, Nakamura Y. ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* 2004; **23**: 7601−10.
6 Kim JK, Estève P-O, Jacobsen SE, Pradhan S. UHRF1 binds G9a and participates in p21 transcriptional regulation in mammalian cells. *Nucleic Acids Res* 2009; **37**: 493–505.
7 Citterio E, Papait R, Nicassio F *et al.* Np95 is a histone-binding protein endowed with ubiquitin ligase activity. *Mol Cell Biol* 2004; **24**: 2526–35.
8 Mousli M, Hopfner R, Abbady AQ *et al.* ICBP90 belongs to a new family of proteins with an expression that is deregulated in cancer cells. *Br J Cancer* 2003; **89**: 120–7.
9 Li XL, Xu JH, Nie JH, Fan SJ. Exogenous expression of UHRF1 promotes proliferation and metastasis of breast cancer cells. *Oncol Rep* 2012; **28**: 375–83.
10 Babbio F, Pistore C, Curti L *et al.* The SRA protein UHRF1 promotes epigenetic crosstalks and is involved in prostate cancer progression. *Oncogene* 2012; **31**: 4878–87.
11 Unoki M, Daigo Y, Koinuma J, Tsuchiya E, Hamamoto R, Nakamura Y. UHRF1 is a novel diagnostic marker of lung cancer. *Br J Cancer* 2010; **103**: 217–22.
12 Unoki M, Kelly JD, Neal DE, Ponder BA, Nakamura Y, Hamamoto R. UHRF1 is a novel molecular marker for diagnosis and the prognosis of bladder cancer. *Br J Cancer* 2009; **101**: 98–105.
13 Kofunato Y, Kumamoto K, Saitou K *et al.* UHRF1 expression is upregulated and associated with cellular proliferation in colorectal cancer. *Oncol Rep* 2012; **28**: 1997–2002.
14 Zhou L, Zhao X, Han Y *et al.* Regulation of UHRF1 by miR-146a/b modulates gastric cancer invasion and metastasis. *FASEB J* 2013; **27**: 4929–39.
15 Tien A, Senbanerjee S, Kulkarni A *et al.* UHRF1 depletion causes a G2/M arrest, activation of DNA damage response and apoptosis. *Biochem J* 2011; **435**: 175–85.
16 Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215–33.
17 Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. *Nat Rev Cancer* 2010; **10**: 389–402.
18 Saadatian Z, Masotti A, Fam ZNS, Alipoor B, Bastami M, Ghaedi H. Single-nucleotide polymorphisms within microRNAs sequences and their 3′UTR target sites may regulate gene expression in gastrointestinal tract cancers. *Iran Red Crescent Med J* 2014; **16**: e16659.
19 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**: 1963–6.
20 Okubo M, Tahara T, Shibata T *et al.* Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. *Helicobacter* 2010; **15**: 524–31.
21 Ramkaran P, Khan S, Phulukdaree A, Moodley D, Chuturgoon AA. miR-146a polymorphism influences levels of miR-146a, IRAK-1, and TRAF-6 in young patients with coronary artery disease. *Cell Biochem Biophys* 2014; **68**: 259–66.
22 Catucci I, Yang R, Verderio P *et al.* Evaluation of SNPs in miR-146a, miR196a2 and miR-499 as low-penetrance alleles in German and Italian familial breast cancer cases. *Hum Mutat* 2010; **31**: E1052–7.
23 Liu Z, Li G, Wei S *et al.* Genetic variants in selected pre-microRNA genes and the risk of squamous cell carcinoma of the head and neck. *Cancer* 2010; **116**: 4753–60.
24 Hu Z, Chen J, Tian T *et al.* Genetic variants of miRNA sequences and non-small cell lung cancer survival. *J Clin Invest* 2008; **118**: 2600–8.
25 Hu Z, Liang J, Wang Z *et al.* Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. *Hum Mutat* 2009; **30**: 79–84.
26 Mittal RD, Gangwar R, George GP, Mittal T, Kapoor R. Investigative role of pre-microRNAs in bladder cancer patients: a case-control study in North India. *DNA Cell Biol* 2011; **30**: 401–6.
27 Xu B, Feng NH, Li PC *et al.* A functional polymorphism in *Pre-miR-146a* gene is associated with prostate cancer risk and mature miR-146a expression *in vivo*. *Prostate* 2010; **70**: 467–72.
28 Guo H, Wang K, Xiong G *et al.* A functional varient in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. *Fam Cancer* 2010; **9**: 599−603.
29 Zeng Y, Sun QM, Liu NN *et al.* Correlation between *pre-miR-146a* C/G polymorphism and gastric cancer risk in Chinese population. *World J Gastroenterol* 2010; **16**: 3578–83.
30 Farzan SF, Karagas MR, Christensen BC, Li Z, Kuriger JK, Nelson HH; New Hampshire Skin Cancer Study. RNASEL and MIR146A SNP–SNP interaction as a susceptibility factor for non-melanoma skin cancer. *PLoS One* 2014; **9**: e93602.
31 Kogo R, Mimori K, Tanaka F, Komune S, Mori M. Clinical significance of miR-146a in gastric cancer cases. *Clin Cancer Res* 2011; **17**: 4277–84.
32 Crew KD, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354–62.
33 Chan AO, Luk JM, Hui WM, Lam SK. Molecular biology of gastric carcinoma: from laboratory to bedside. *J Gastroenterol Hepatol* 1999; **14**: 1150–60.
34 Lorenzato M, Caudroy S, Bronner C *et al.* Cell cycle and/or proliferation markers: what is the best method to discriminate cervical high-grade lesions? *Hum Pathol* 2005; **36**: 1101–7.
35 Crnogorac-Jurcevic T, Gangeswaran R, Bhakta V *et al.* Proteomic analysis of chronic pancreatitis and pancreatic adenocarcinoma. *Gastroenterology* 2005; **129**: 1454–63.
36 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 U S A* 2008; **105**: 7269–74.
37 Jazdzewski K, Liyanarachchi S, Swierniak M *et al.* Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer. *Proc Natl Acad Sci U S A* 2009; **106**: 1502–5.
38 Akkız H, Bayram S, Bekar A, Akgöllü E, Üsküdar O, Sandıkçı M. No association of pre-microRNA-146a rs2910164 polymorphism and risk of hepatocellular carcinoma development in Turkish population: a case-control study. *Gene* 2011; **486**: 104–9.
39 Senses KM, Gonen M, Barutcu AR *et al.* Cancer-testis gene expression is associated with the methylenetetrahydrofolate reductase 677 C>T polymorphism in non-small cell lung carcinoma. *BMC Med Genet* 2013; **14**: 97.
40 Zhang Y, Huang Z, Zhu Z *et al.* Upregulated UHRF1 promotes bladder cancer cell invasion by epigenetic silencing of KiSS1. *PLoS One* 2014; 9: e104252.