Journal of Human Genetics (2014) 59, 141–144

www.nature.com/jhg

ORIGINAL ARTICLE

Association of miR-146a gene polymorphism with risk of nasopharyngeal carcinoma in the central-southern Chinese population

Guo-Liang Huang[1,2,6], Mei-Ling Chen[3,6], Ya-Zhen Li[3,6], Yan Lu[1,2], Xing-Xiang Pu[4], Yu-Xiang He[5], Shu-Yin Tang[3], Hua Che[3], Ying Zou[1,2], Congcong Ding[1,2] and Zhiwei He[1,2]

This case-control study focused on estimating the association between miR-146a polymorphism and risk of nasopharyngeal carcinoma (NPC) in central-south China. In total, 160 patients with NPC and 200 healthy controls in central-south China were genotyped using polymerase chain reaction–restriction fragment length polymorphism assay. Chi-square test was used to assess the different distribution of miR-146a polymorphism between NPC patients and controls; and logistic regression analysis was applied to analyze the associations between miR-146a polymorphism with cancer risk in different contrast models. Significant differences between NPC patients and controls were found in genotype ($P = 0.033$ for GG versus CG versus CC; and odds ratio (OR) = 0.568, 95% confidence interval (CI) = 0.354–0.912, $P = 0.019$ for CG versus CC; and OR = 0.503, 95% CI = 0.261–0.971, $P = 0.041$ for CG versus CC; and OR = 0.564, 95% CI = 0.360–0.884, $P = 0.012$ for GG + CG versus CC, respectively) and allelic analysis ($P = 0.025$ for G versus C). Our findings suggested that polymorphism of mir-146a was associated with NPC in the central-southern Chinese population.
Journal of Human Genetics (2014) **59,** 141–144; doi:10.1038/jhg.2013.135; published online 16 January 2014

Keywords: cancer risk; mir-146a; nasopharyngeal carcinoma; polymorphism; SNP

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a highly invasive malignant tumor that is common in Southeastern China and South East Asia.[1] The multifactorial etiology of NPC includes genetic susceptibility owing to the distinct geographical distribution, Epstein–Barr virus (EBV) infection and exposure to chemical carcinogens in environment and dietary.[2] Previous genetic studies reported that several genes were associated with the risk of NPC.[3–5]

MicroRNAs (miRNAs) are endogenous ~22 nucleotides non-coding RNAs and function as tumor suppressors or oncogenes in cancer.[6] miR-146a function as a tumor suppressor in most cases.[7] Mouse miR-146a knockout models suggested that miR-146a played a key role as a molecular brake on oncogenic transformation.[8] In breast cancer, enforced miR-146a expression suppressed NF-κB activity and impaired invasion and migration capacity of cancer cells.[9] A second study indicated that the inhibitory effect of tumor suppressor p53-binding protein-1 on NF-κB activity, cell proliferation, invasion and metastasis in breast cancer was mediated by miR-146a.[10] TRAIL-induced miR-146a expression impaired CXCR4-mediated breast cancer migration.[11] In gastric cancer, miR-146a play a role as a metastasis suppressor by targeting WASF2,[12] UHRF1[13] and L1CAM.[14] Upregulation of miR-146a expression inhibited NF-κB activity induced by lysophosphatidic acid or celastrol in gastric cancer cells.[15,16] The tumor suppressor role of miR-146a was also found in non-small cell lung cancer,[17] papillary thyroid tumor,[18] acute myeloid leukemia,[19] castration-resistant prostate cancer[20] and glioma.[21] Nevertheless, miR-146a was found to promote proliferation in cervical cancer[22] and enhance angiogenic activity of endothelial cells in hepatocellular carcinoma.[23]

The role of miR-146a on NPC was seldom characterized. The miR-146a promoter was found to be completely unmethylated, but lack of euchromatic histone modification in the EBV-positive NPC cell line C666-1.[24] A minimal amount of pre-miR-146a and negative expression of mature miR-146a was detected in C666-1 cells. The expression levels of miR-146a was significantly increased and positively correlated with EBV-encoded latent membrane protein 1 (LMP1) in NPC tissues.[25] LMP1 was reported to activate the expression of miR-146a via NF-κB.[26,27] miR-146a was downregulated by EBV-encoded EBNA2 in B lymphoma cells.[28] Accordingly, miR-146a may be an important mediator in EBV-associated carcinogenesis.

[1]Sino-American Cancer Research Institute, Guangdong Medical College, Dongguan, China; [2]Key Laboratory for Medical Molecular Diagnostics of Guangdong Province, Dongguan, China; [3]Institute Of Laboratory Medicine, Guangdong Medical College, Dongguan, China; [4]Department of Medical Oncology, Hunan Tumor Hospital, Changsha, China and [5]Department of Oncology, Central South University Xiangya Hospital, Changsha, China
[6]These authors contributed equally to this work.
Correspondence: Professor Z He, Sino-American Cancer Research Institute, Guangdong Medical College, No. 1 Xincheng Road, Dongguan 523808, China.
E-mail: zhiweihe688@yahoo.com
Received 8 August 2013; revised 22 November 2013; accepted 26 November 2013; published online 16 January 2014

Previous studies showed that single nucleotide polymorphisms (SNPs) in miR-146a gene could deregulate the expression of miR-146a and thereby affect its function. A study in systemic lupus erythematosus indicated that rs57095329 in the promoter region of miR-146a modulated its expression and was associated with risk for the disease.[29] The rs2910164 within the stem-loop region of the pre-miR-146a was mainly investigated SNP in miR-146a. The rs2910164 was found to affect the expression of miR-146a.[30] GC heterozygote of this SNP could produce three mature miRNAs: miR-146a*G and miR-146a*C from the passenger strand, and miR-146a from the leading strand.[31] They are proposed to regulate many genetic processes with distinct sets of target genes.

Associations between rs2910164 in miR-146a gene with risk or prognosis of cancer were found in several types of cancer, including non-small cell lung cancer,[32] esophageal squamous cell carcinoma,[33] gastric cancer,[34] glioma[35] oral squamous cell carcinoma,[36] cervical cancer[37] and so on. In a recent study, SNP rs2910164 in miR-146a gene was found to be associated with the risk for NPC in the southern Chinese population in Hong Kong.[25] In this study, we genotyped the frequency of SNP rs2910164 in miR-146a and evaluated the association with NPC risk in a case-control study of the central-southern Chinese population.

MATERIALS AND METHODS

Study population

The hospital-based case-control study included 160 patients with nasopharyngeal cancer and 200 healthy individuals at Hunan Tumor Hospital between April 2011 and October 2011. All the cancer patients were diagnosed by histopathology or imaging evidence and received no treatment before the blood drawing. The control participants were genetically unrelated cancer-free individuals who underwent routine medical examination in the same hospital. No significant differences with the age and gender were found between patients and controls (mean ± s.d. = 46.2 ± 11.3 and mean ± s.d. = 44.7 ± 10.4, respectively, Table 1). All participants were Changsha natives (Changsha, central-southern China) or the surrounding regions. Informed consent was obtained from all subjects for the use of their blood samples. This study protocol was approved by the institutional review board.

Genotyping assay

Genomic DNA was extracted from peripheral blood samples on the basis of standard procedures (Tiangen Biotech, Beijing, China). The genotype of SNP rs2910164 in miR-146a was detected by polymerase chain reaction (PCR)–restriction fragment length polymorphism assay according to Hu *et al*.[38] The PCR reaction contained 5 pmol of each primer, 1× GoTaq Master Mix (Promega, Beijing, China) and 2.0 μl extracted DNA in a total volume of 15 μl. The PCR condition included an initial melting step at 95 °C for 5 min, then 30 cycles at 95 °C for 30 s, at 60 °C for 30 s and at 72 °C for 40 s, and a final extension step at 72 °C for 10 min. The fragments were amplified by using the forward primer (5′-CAT GGG TTG TGT CAG TGT CAG AG CT-3′) and reverse primer (5′-TGC CTT CTG TCT CCA GTC TTC CAA-3′). Then the PCR products were digested by Saclat 37 °C for 3 h. After that, the cleaved products were separated on polyacrylamide gel electrophoresis and identified by ethidium bromide staining. According to the restriction site of the enzyme, GG genotype was cleaved to be one band (147 bp); CC to be two bands (122 and 25 bp); GC to be three bands (147, 122 and 25 bp). PCR products were confirmed by DNA sequencing validation in about 5% of the samples.

Statistical analysis

Genotype frequencies in control group were detected for the Hardy–Weinberg equilibrium using chi-square test. The diverse characteristics between patients and controls were analyzed by *t*-test or chi-square test. The different distribution of the genotype and allele frequencies was evaluated by chi-square test. The association between miR-146a polymorphism and nasopharyngeal cancer risk was estimated by calculating the odds ratios (ORs) and 95% confidence intervals (CIs), using the multivariate logistic regression analysis adjusted by age and gender. All statistical tests were performed with SPSS 16.0 software. All results were statistically significant at $P<0.05$.

RESULTS

Characteristics of patients with NPC and healthy controls

The case-control study recruited 360 samples including 160 patients with nasopharyngeal cancer and 200 healthy individuals. Main characteristics of the study subjects were presented in Table 1. There were no statistically significant differences between cases and controls, in terms of age ($P=0.555$, Table 1) and gender ($P=0.146$, Table 1). Most of the patients were in the late clinical stage (Table 1).

Distribution of genotype and allele frequencies and their associations with risk of NPC

Table 2 showed the distribution of genotype and allele frequencies of miR-146a polymorphism in the patients and controls. Genotype frequencies for miR-146a polymorphism were in agreement with Hardy–Weinberg equilibrium in the controls ($P=0.123$). The frequencies of the GG, CG and CC genotypes were 14.4, 45.6 and 40.0%, respectively, among the patients, with 18.0, 55.0 and 27.0%, respectively, among the controls; and the allele frequencies of G and C were 37.2 and 62.8% in the patients, with 45.5 and 54.5% in the controls. Significant differences were found between patients and controls in genotype ($P=0.033$ for GG versus CG versus CC; OR = 0.568, 95% CI = 0.354–0.912, $P=0.019$ for CG versus CC; OR = 0.503, 95% CI = 0.261–0.971, $P=0.041$ for GG versus CC; and OR = 0.564, 95% CI = 0.360–0.884, $P=0.012$ for GG + CG versus CC, respectively, Table 2) and allelic analysis ($P=0.025$, Table 2).

Table 1 Characteristics of NPC Patients and Controls

characteristics	*Patients*	*Controls*	P-*value*
Mean age (mean ± s.d.)	46.2 ± 11.3	44.7 ± 10.4	0.196
Age			
<45	77 (48.1)	90 (45.0)	0.555
⩾45	83 (51.9)	110 (55.0)	
Gender			
Male	117 (73.1)	132 (66.0)	0.146
Female	43 (26.9)	68 (34.0)	
T stage			
T1 + T2	56 (35.0)	—	
T3 + T4	104 (65.0)	—	
N stage			
N0	9 (5.6)	—	
N1 + N2 + N3	151 (94.4)	—	
Clinical stage			
I + II	11 (6.9)	—	
III + IV	149 (93.1)	—	
Metastasis			
No	148 (94.3)	—	
Yes	9 (5.7)	—	

Abbreviation: s.d., standard deviation.
The sum of various characteristics does not equal because of the unavailable data.

Table 2 Genotype and Allele Distribution of rs2910164 in Patients and Controls

Polymorphism	*Patient*	*Control*	P-*value*[a]	*Crude OR (95% CI)*	P-*value*	*Adjusted OR (95% CI)*	P-*value*[b]
rs2910164 C>G							
Genotype							
GG	23 (14.4)	36 (18.0)	0.033				
CG	73 (45.6)	110 (55.0)					
CC	64 (40.0)	54 (27.0)					
CG versus CC			0.015	0.560 (0.351-0.894)	0.015	0.568 (0.354-0.912)	0.019
GG versus CC			0.056	0.539 (0.285-1.019)	0.057	0.503 (0.261-0.971)	0.041
GG + CG versus CC			0.009	0.555 (0.356-0.865)	0.009	0.564 (0.360-0.884)	0.012
Allele							
G	119 (37.2)	182 (45.5)	0.025				
C	201 (62.8)	218 (54.5)					

Abbreviations: CI, confident interval; OR, odd ratio.
[a]*P*-values were calculated from two-sided chi-square tests.
[b]Data were calculated by logistic regression with adjustment for age and gender.

DISCUSSION

Studies about the association between SNP rs2910164 in miR-146a and cancer susceptibility provided contradictory results regarding the effect of SNP rs2910164 on cancer. A protective role of rs2910164 C allele or CC genotype on cancer was reported in several studies. The rs2910164 C allele or CC genotype was associated with decreased risk for esophageal cancer, cervical cancer, prostate cancer, hepatocellular carcinoma and colorectal cancer.[39–41] The rs2910164 GC/CC genotypes conferred a significantly reduced risk of recurrence in bladder cancer and a higher survival rate in gastric cancer.[34,42]

However, the rs2910164 C allele or CC genotype was also reported to contribute to an increased susceptibility to cancer, such as head and neck cancer,[43] glioma[35] and NPC.[25] The rs2910164 GC/CC genotypes had significantly worse overall, disease-specific and disease-free survival in squamous cell carcinoma of the oropharynx and a worse recurrence-free survival in non-small cell lung cancer.[44,45] These results suggested a promotive role of rs2910164 C allele or CC genotype on cancer. As in the case of our study, the results suggested that rs2910164 C allele or CC genotype contribute to an increased risk of NPC in the central-southern Chinese population.

The different effect of SNP rs2910164 on cancer might derive from the inconsistent regulation of SNP rs2910164 on miR-146a. Higher levels of miR-146a were associated with the CC genotype in several disease.[36,37,46,47] Whereas miR-146a expression in GG cases was higher in other studies.[48–50] The effect of SNP rs2910164 seems more complicated because the GC heterozygote of this SNP could produce three mature miRNAs: one from the leading strand (miR-146a), and two from the passenger strand (miR-146a*G and miR-146a*C).[31] These three miRNAs were proposed to modulate many genetic processes with distinct sets of target genes. Therefore, by producing inconsistent levels of and different kind of mature miRNAs, the rs2910164 may affect cancer risk in contradictory ways.

Both the previous study on NPC by Lung *et al.*[25] and our data suggested that the rs2910164 C allele or CC genotype was associated with the increase risk of the cancer. The gender distribution was similar in both the healthy controls and NPC patients between our study and the data of Lung *et al.*[25] in Hong Kong. Both study populations were Chinese. Whereas the age distribution in our study was younger in both the healthy controls and NPC patients. Similar to the data of Lung *et al.*[25] in Hong Kong, a much higher distribution of CC genotype which accounted for 27% of the healthy controls and 40.0% of the NPC patients was found in our data, when compared with the Western population in which only 6% with CC genotype.[30] The CC genotype frequency of rs2910164 in Hong Kong was 36.2% in the matched controls group, 39.1% in the healthy elderly controls group and 51.1% in the NPCs group, respectively. Among the three areas, the NPC incidence rate was greatest in Hong Kong, then our locality central-southern China and lowest in the Western country. The CC genotype frequency of rs2910164 might in part explain the NPC incidence rate.

The exact role of miR-146a in NPC was not clarified. Lung *et al.*[25] found higher expression of miR-146a, which was correlated with LMP1, but not with rs2910164 polymorphism in NPC. Cell proliferation was not affected by over-expression of miR-146a in normal nasopharyngeal epithelial cell line NP69. The result indicated that miR-146a might modulate NPC progression by other means, such as apoptosis, cell invasion, migration and the interaction with LMP1. Further functional study should be carried out to confirm these hypotheses. Since the sample size of this case-control study and Lung *et al.* was relatively small, further large sample studies were needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Natural Science Foundation of Guangdong Province (to ZH), National Natural Science Foundation of China (no: 81102048 to G-LH), Science and Technology Innovation Fund of Guangdong Medical College (no: STIF201108 to ZH), and Medical Science Research Foundation of Guangdong Province (no: B2011232 to G-LH).

Author contributions: Experiment and data analysis: Guo-Liang Huang, Mei-Ling Chen, Ya-Zhen Li, Yan Lu, Xing-Xiang Pu; sample and data collection: Xing-Xiang Pu, Yu-Xiang He, Shu-Yin Tang, Hua Che, Ying Zou, Cong-cong Ding; statistics and draft writing: Guo-Liang Huang, Mei-Ling Chen, Ya-Zhen Li; conception, design and editing: Guo-Liang Huang, Zhiwei He.

1 Parkin, D. M., Pisani, P. & Ferlay, J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int. J. Cancer* **80,** 827–841 (1999).
2 Tao, Q. & Chan, A. T. Nasopharyngeal carcinoma: molecular pathogenesis and therapeutic developments. *Expert. Rev. Mol. Med.* **9,** 1–24 (2007).
3 Feng, B. J., Huang, W., Shugart, Y. Y., Lee, M. K., Zhang, F., Xia, J. C. *et al.* Genome-wide scan for familial nasopharyngeal carcinoma reveals evidence of linkage to chromosome 4. *Nat. Genet.* **31,** 395–399 (2002).

4 Zhou, G., Zhai, Y., Cui, Y., Zhang, X., Dong, X., Yang, H. *et al.* MDM2 promoter SNP309 is associated with risk of occurrence and advanced lymph node metastasis of nasopharyngeal carcinoma in Chinese population. *Clin. Cancer Res.* **13,** 2627–2633 (2007).
5 Zhang, Y., Zhang, H., Zhai, Y., Wang, Z., Ma, F., Wang, H. *et al.* A functional tandem-repeats polymorphism in the downstream of TERT is associated with the risk of nasopharyngeal carcinoma in Chinese population. *BMC Med.* **9,** 106. doi:10.1186/1741-7015-9-106 (2011).
6 Esquela-Kerscher, A. & Slack, F. J. Oncomirs – microRNAs with a role in cancer. *Nat. Rev. Cancer* **6,** 259–269 (2006).
7 Labbaye, C. & Testa, U. The emerging role of MIR-146A in the control of hematopoiesis, immune function and cancer. *J. Hematol. Oncol.* **5,** 13. doi:10.1186/1756-8722-5-13 (2012).
8 Boldin, M. P., Taganov, K. D., Rao, D. S., Yang, L., Zhao, J. L., Kalwani, M. *et al.* miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J. Exp. Med.* **208,** 1189–1201 (2011).
9 Bhaumik, D., Scott, G. K., Schokrpur, S., Patil, C. K., Campisi, J. & Benz, C. C. Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. *Oncogene* **27,** 5643–5647 (2008).
10 Li, X., Xu, B., Moran, M. S., Zhao, Y., Su, P., Haffty, B. G. *et al.* 53BP1 functions as a tumor suppressor in breast cancer via the inhibition of NF-kappaB through miR-146a. *Carcinogenesis* **33,** 2593–2600 (2012).
11 Wang, D., Liu, D., Gao, J., Liu, M., Liu, S., Jiang, M. *et al.* TRAIL-induced miR-146a expression suppresses CXCR4-mediated human breast cancer migration. *FEBS J.* **280,** 3340–3353 (2013).
12 Yao, Q., Cao, Z., Tu, C., Zhao, Y., Liu, H. & Zhang, S. MicroRNA-146a acts as a metastasis suppressor in gastric cancer by targeting WASF2. *Cancer Lett.* **335,** 219–224 (2013).
13 Zhou, L., Zhao, X., Han, Y., Lu, Y., Shang, Y., Liu, C. *et al.* Regulation of UHRF1 by miR-146a/b modulates gastric cancer invasion and metastasis. *FASEB J.* **27,** 4929–4939 (2013).
14 Hou, Z., Yin, H., Chen, C., Dai, X., Li, X., Liu, B. *et al.* microRNA-146a targets the L1 cell adhesion molecule and suppresses the metastatic potential of gastric cancer. *Mol. Med. Rep.* **6,** 501–506 (2012).
15 Crone, S. G., Jacobsen, A., Federspiel, B., Bardram, L., Krogh, A., Lund, A. H. *et al.* microRNA-146a inhibits G protein-coupled receptor-mediated activation of NF-kappaB by targeting CARD10 and COPS8 in gastric cancer. *Mol. Cancer* **11,** 71. doi:10.1186/1476-4598-11-71 (2012).
16 Sha, M., Ye, J., Zhang, L. X., Luan, Z. Y. & Chen, Y. B. Celastrol induces apoptosis of gastric cancer cells by miR-146a inhibition of NF-kappaB activity. *Cancer Cell Int.* **13,** 50. doi:10.1186/1475-2867-13-50 (2013).
17 Chen, G., Umelo, I. A., Lv, S., Teugels, E., Fostier, K., Kronenberger, P. *et al.* miR-146a inhibits cell growth, cell migration and induces apoptosis in non-small cell lung cancer cells. *PLoS One* **8,** e60317 (2013).
18 Zhang, X., Li, D., Li, M., Ye, M., Ding, L., Cai, H. *et al.* MicroRNA-146a targets PRKCE to modulate papillary thyroid tumor development. *Int. J. Cancer* **134,** 257–267 (2014).
19 Spinello, I., Quaranta, M. T., Riccioni, R., Riti, V., Pasquini, L., Boe, A. *et al.* MicroRNA-146a and AMD3100, two ways to control CXCR4 expression in acute myeloid leukemias. *Blood Cancer J.* **1,** e26 (2011).
20 Xu, B., Wang, N., Wang, X., Tong, N., Shao, N., Tao, J. *et al.* 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,** 1171–1178 (2012).
21 Mei, J., Bachoo, R. & Zhang, C. L. MicroRNA-146a inhibits glioma development by targeting Notch1. *Mol. Cell Biol.* **31,** 3584–3592 (2011).
22 Wang, X., Tang, S., Le, S. Y., Lu, R., Rader, J. S., Meyers, C. *et al.* Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. *PLoS One* **3,** e2557 (2008).
23 Zhu, K., Pan, Q., Zhang, X., Kong, L. Q., Fan, J., Dai, Z. *et al.* MiR-146a enhances angiogenic activity of endothelial cells in hepatocellular carcinoma by promoting PDGFRA expression. *Carcinogenesis* **34,** 2071–2079 (2013).
24 Szenthe, K., Koroknai, A., Banati, F., Bathori, Z., Lozsa, R., Burgyan, J. *et al.* The 5' regulatory sequences of active miR-146a promoters are hypomethylated and associated with euchromatic histone modification marks in B lymphoid cells. *Biochem. Biophys. Res. Commun.* **433,** 489–495 (2013).
25 Lung, R. W., Wang, X., Tong, J. H., Chau, S. L., Lau, K. M., Cheng, S. H. *et al.* A single nucleotide polymorphism in microRNA-146a is associated with the risk for nasopharyngeal carcinoma. *Mol. Carcinog.* **52** (Suppl 1), 28–38 (2013).
26 Motsch, N., Pfuhl, T., Mrazek, J., Barth, S. & Grasser, F. A. Epstein-Barr virus-encoded latent membrane protein 1 (LMP1) induces the expression of the cellular microRNA miR-146a. *RNA Biol.* **4,** 131–137 (2007).
27 Cameron, J. E., Yin, Q., Fewell, C., Lacey, M., McBride, J., Wang, X. *et al.* Epstein-Barr virus latent membrane protein 1 induces cellular MicroRNA miR-146a, a modulator of lymphocyte signaling pathways. *J. Virol.* **82,** 1946–1958 (2008).
28 Rosato, P., Anastasiadou, E., Garg, N., Lenze, D., Boccellato, F., Vincenti, S. *et al.* Differential regulation of miR-21 and miR-146a by Epstein-Barr virus-encoded EBNA2. *Leukemia* **26,** 2343–2352 (2012).
29 Luo, X., Yang, W., Ye, D. Q., Cui, H., Zhang, Y., Hirankarn, N. *et al.* A functional variant in microRNA-146a promoter modulates its expression and confers disease risk for systemic lupus erythematosus. *PLoS Genet.* **7,** e1002128 (2011).
30 Jazdzewski, K., Murray, E. L., Franssila, K., Jarzab, B., Schoenberg, D. R. & de la Chapelle, A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc. Natl Acad. Sci. USA* **105,** 7269–7274 (2008).
31 Jazdzewski, K., Liyanarachchi, S., Swierniak, M., Pachucki, J., Ringel, M. D., Jarzab, B. *et al.* Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer. *Proc. Natl Acad. Sci. USA* **106,** 1502–1505 (2009).
32 Vinci, S., Gelmini, S., Pratesi, N., Conti, S., Malentacchi, F., Simi, L. *et al.* Genetic variants in miR-146a, miR-149, miR-196a2, miR-499 and their influence on relative expression in lung cancers. *Clin. Chem. Lab. Med.* **49,** 2073–2080 (2011).
33 Guo, H., Wang, K., Xiong, G., Hu, H., Wang, D., Xu, X. *et al.* A functional variant in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. *Fam. Cancer* **9,** 599–603 (2010).
34 Ahn, D. H., Rah, H., Choi, Y. K., Jeon, Y. J., Min, K. T., Kwack, K. *et al.* Association of the miR-146aC>G, miR-149 T>C, miR-196a2T>C, and miR-499 A>G polymorphisms with gastric cancer risk and survival in the Korean population. *Mol. Carcinog.* **52** (Suppl 1), 39–51 (2013).
35 Permuth-Wey, J., Thompson, R. C., Burton Nabors, L., Olson, J. J., Browning, J. E., Madden, M. H. *et al.* A functional polymorphism in the pre-miR-146a gene is associated with risk and prognosis in adult glioma. *J. Neurooncol.* **105,** 639–646 (2011).
36 Hung, P. S., Chang, K. W., Kao, S. Y., Chu, T. H., Liu, C. J. & Lin, S. C. Association between the rs2910164 polymorphism in pre-mir-146a and oral carcinoma progression. *Oral. Oncol.* **48,** 404–408 (2012).
37 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.* **122,** 33–37 (2011).
38 Hu, Z., Chen, J., Tian, T., Zhou, X., Gu, H., Xu, L. *et al.* Genetic variants of miRNA sequences and non-small cell lung cancer survival. *J. Clin. Invest.* **118,** 2600–2608 (2008).
39 Yin, Z., Yan, L., Cui, Z., Li, X., Ren, Y. & Zhou, B. Effects of common polymorphisms rs2910164 in miR-146a and rs3746444 in miR-499 on cancer susceptibility: a meta-analysis. *Mol. Biol. Rep.* **40,** 3003–3013 (2013).
40 He, B., Pan, Y., Cho, W. C., 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* **7,** e49032 (2012).
41 Ma, L., Zhu, L., Gu, D., Chu, H., Tong, N., Chen, J. *et al.* A genetic variant in miR-146a modifies colorectal cancer susceptibility in a Chinese population. *Arch. Toxicol.* **87,** 825–833 (2013).
42 Wang, M., Chu, H., Li, P., Yuan, L., Fu, G., Ma, L. *et al.* Genetic variants in miRNAs predict bladder cancer risk and recurrence. *Cancer Res.* **72,** 6173–6182 (2012).
43 Orsos, Z., Szanyi, I., Csejtei, A., Gerlinger, I., Ember, I. & Kiss, I. Association of pre-miR-146a rs2910164 Polymorphism with the Risk of Head and Neck Cancer. *Anticancer Res.* **33,** 341–346 (2013).
44 Guan, X., Sturgis, E. M., Song, X., Liu, Z., El-Naggar, A. K., Wei, Q. *et al.* Pre-microRNA variants predict HPV16-positive tumors and survival in patients with squamous cell carcinoma of the oropharynx. *Cancer Lett.* **330,** 233–240 (2013).
45 Yoon, K. A., Yoon, H., Park, S., Jang, H. J., Zo, J. I., Lee, H. S. *et al.* The prognostic impact of microRNA sequence polymorphisms on the recurrence of patients with completely resected non-small cell lung cancer. *J. Thorac. Cardiovasc. Surg.* **144,** 794–807 (2012).
46 Shen, J., Ambrosone, C. B., DiCioccio, R. A., Odunsi, K., Lele, S. B. & Zhao, H. A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. *Carcinogenesis* **29,** 1963–1966 (2008).
47 Ramkaran, P., Khan, S., Phulukdaree, A., Moodley, D. & Chuturgoon, A. A. miR-146a polymorphism influences levels of miR-146a, IRAK-1, and TRAF-6 in young patients with coronary artery disease. *Cell Biochem. Biophys.* (e-pub ahead of print 22 June 2013; doi:10.1007/s12013-013-9704-7).
48 Zhou, Q., Hou, S., Liang, L., Li, X., Tan, X., Wei, L. *et al.* MicroRNA-146a and Ets-1 gene polymorphisms in ocular Behcet's disease and Vogt-Koyanagi-Harada syndrome. *Ann. Rheum. Dis.* **73,** 170–176 (2012).
49 Jiang, H., He, X., Li, J., Xie, Q., Lin, J. & Chang, Y. Association of a single-nucleotide polymorphism within the miR-146a gene with susceptibility for acute-on-chronic hepatitis B liver failure. *Immunogenetics* **65,** 257–263 (2013).
50 Xu, B., Feng, N. H., Li, P. C., Tao, J., Wu, D., Zhang, Z. D. *et al.* A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. *Prostate* **70,** 467–472 (2010).