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BRIEF ARTICLE

Evaluation of SNPs in miR-196-a2, miR-27a and miR-146a as risk factors of colorectal cancer

Renata Hezova, Alena Kovarikova, Julie Bienertova-Vasku, Milana Sachlova, Martina Redova, Anna Vasku, Marek Svoboda, Lenka Radova, Igor Kiss, Rostislav Vyzula, Ondrej Slaby

Renata Hezova, Alena Kovarikova, Milana Sachlova, Martina Redova, Marek Svoboda, Igor Kiss, Rostislav Vyzula, Ondrej Slaby, Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech
Julie Bienertova-Vasku, Anna Vasku, Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech
Martina Redova, Ondrej Slaby, Central European Institute of Technology, Masaryk University, 625 00 Brno, Czech
Lenka Radova, Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and Palacky University Affiliated Hospital Olomouc, 775 15 Olomouc, Czech
Author contributions: Hezova R and Kovarikova A performed DNA purifications and single nucleotide polymorphism analysis; Sachlova M, Vasku A, Svoboda M, Kiss I and Vyzula R collected DNA samples and clinical data from patients and controls involved in the study; Radova L performed statistical evaluation of the collected data; Bienertova-Vasku J, Redova M and Slaby O participated in manuscript preparation; and Slaby O designed the study, performed the data analysis and interpretation, and critically revised the manuscript.
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Correspondence to: Ondrej Slaby, PhD, Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech. slaby@mou.cz
Telephone: +420-5-43136902 Fax: +420-5-43136902
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Abstract

AIM: To investigate whether selected single nucleotide polymorphisms (SNPs) in miR-196a2, miR-27a and miR-146a genes are associated with sporadic colorectal cancer (CRC).

METHODS: In order to investigate the effect of these SNPs in CRC, we performed a case-control study of 197 cases of sporadic CRC and 212 cancer-free controls originating from the Central-European Caucasian population using TaqMan Real-Time polymerase chain reaction and allelic discrimination analysis.

RESULTS: The genotype and allele frequencies of SNPs were compared between the cases and the controls. None of the performed analysis showed any statistically significant results.

CONCLUSION: Our data suggest a lack of association between rs11614913, rs895819 and rs2910164 and colorectal cancer risk in the Central-European Caucasian population, a population with an extremely high incidence of sporadic colorectal cancer.

Key words: Association study; Colorectal cancer; MicroRNA; Single nucleotide polymorphism

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INTRODUCTION

Sporadic colorectal cancer represents a typical multifacto-

rial disease with an intense crosstalk of the genetic background with the environment, including lifestyle habits and diet. Certain populations present higher rates of sporadic colorectal cancer, independently of diet and lifestyle habits than others[1], which supports the hypothesis that individual genetic background is involved in the etiopathogenesis of the disease. An extremely high incidence of colorectal cancer[1] has been repeatedly reported for the Central-European Caucasian population, significantly exceeding the peak incidence observed in the United States and other developed countries[2]. This population is, therefore, highly likely to carry a strong genetic predisposition to sporadic colorectal cancer and could be a good model population for sporadic colorectal cancer.

MicroRNAs (miRNAs) are short non-coding RNAs, 18 to 25 nucleotides in length, which regulate gene expression[3]. Single nucleotide polymorphisms (SNPs) may occur at the level of the miRNA biogenesis pathway genes, pri-miRNA, pre-miRNA or mature miRNA sequences. Such polymorphisms may be functional with regard to the biogenesis and actions of the mature miRNA. Specific SNPs are located at predicted miRNA target sites within 3' of untranslated regions of mRNAs. These SNPs have the potential to affect the efficiency of miRNA binding at their target sites as well as to create or disrupt binding sites. Resulting gene dysregulation may involve changes in phenotype and may eventually prove critical for the susceptibility to and the onset of cancer, as well as for prognosis and therapy response prediction[1].

The most frequently studied miRNA-associated SNP in cancer is rs11614913 in the pre-miRNA region of miR-196-a2. Hu *et al*[4] observed the association of the rs11614913: T > C variant genotype with a significantly increased risk of breast cancer [odds ratio (OR) 1.23; 95% confidence interval (CI): 1.02-1.48]. A number of case-control studies were consequently performed in breast[5,6], lung[7,8], gastric[9], esophageal[10], hepatocellular[11] and head and neck cancer[12]. More recently, two contradictory studies were published evaluating rs11614913 as a potential risk factor for colorectal cancer in the Chinese population (T *vs* C allele-OR 1.320; CI: 1.056-1.649, P = 0.014[13] *vs* OR 1.065; CI: 0.803-1.414, P = 0.665[14]). SNP rs895819, located in the terminal loop of a pre-miR-27a oncogene, was initially evaluated in familial breast cancer, whereas the G allele was associated with reduced familial breast cancer risk (P = 0.0215). The opposite of this association was observed by Sun *et al*[15] in a gastric cancer case-control study where subjects with variant genotypes (AG + GG) showed a significantly increased risk of gastric cancer relative to AA carriers (OR 1.48; 95% CI: 1.06-2.05; P = 0.019). AG to C SNP (rs2910164) located within the sequence of the miR-146a precursor was first studied by Shen *et al*[16] due to the fact that predicted miR-146a target genes include BRCA1 and BRCA2, i.e., key breast and ovarian cancer susceptibility genes. Breast and ovarian cancer patients who had at least one miR-146a variant allele were diagnosed at an earlier age. Subsequently, the distribution of the miR-146a polymorphism rs2910164 was evaluated in breast[6], esophageal[17], hepatocellular[18] and thyroid cancer[19].

Thus, a significant association with the risk of various types of solid cancers, with the exception of colorectal cancer, has been repeatedly reported for SNPs: rs11614913 in miR-196-a2, rs895819 in hsa-miR-27a and rs2910164 in miR-146a; consequently, we decided to perform a case-control study evaluating these three SNPs and the risk of sporadic colorectal cancer in a Central-European Caucasian population.

MATERIALS AND METHODS

Patients and controls

The study included patients with newly diagnosed sporadic colorectal cancer treated at the Masaryk Memorial Cancer Institute, Czech Republic between January 2008 and December 2010. The patient cohort consisted of 197 subjects [105 men, 92 women; age (mean ± SD): 63 ± 9 years] with histologically confirmed colorectal adenocarcinomas, whereas the control cohort included a total of 202 cancer-free blood donor volunteers recruited from the same institute with a similar age distribution (93 men, 109 women; mean age: 65 ± 14 years) and no previous history of any type of cancer. Due to its invasiveness, colonoscopy was not performed to exclude colorectal cancer (CRC) in the control cohort; however, all subjects were symptom free and no anemia was present. All study subjects were Caucasian. The hospital ethical committee approved the study and all study subjects supplied a written informed consent which was subsequently archived.

DNA isolation and genotyping

Genomic DNA was isolated from the full peripheral blood using the MagNA Pure DNA Isolator (Roche). DNA concentration was measured on the Nanodrop ND-1000 (NanoDrop Technologies, Inc.). For analysis of rs11614913 in miR-196-a2, rs895819 in hsa-miR-27a and rs2910164 in miR-146a, Real-Time polymerase chain reaction (PCR) allelic discrimination was performed on Step-One Real-Time PCR (Applied Biosystems, United States) using standard TaqMan genotyping assays according to the manufacturer's instructions. In brief, probes, primers and TaqMan universal PCR Master Mix were obtained from Applied Biosystems. A reaction solution of 10 μL contained 0.5 μL TaqMan Genotyping Assay mix (consisting of 20X Mix of unlabeled PCR primers and TaqMan minor groove binder probe, 6-carboxyfluorescein and VIC dye-labeled), 8 μL of PCR mixture reagent and 10 ng of genomic DNA. Reactions were run according to the manufacturer's instructions. The PCR consisted of pre-PCR read at 60 ℃ for 30 s, holding stage at 95 ℃ for 10 min, 50 cycles of denaturing at 92 ℃ for 15 s, annealing 60 ℃ for 1 min 30 s and post-PCR read at 60 ℃ for 30 s.

Statistical analysis

The Hardy-Weinberg equilibrium was tested for each

Table 1 Logistic regression analysis of genotype frequencies of single nucleotide polymorphisms rs11614913, rs895819 and rs2910164 in colorectal cancer cases and controls in the Czech population

		Control		CRC				
		n	%	*n*	%	OR[1]	95% CI	*P* value
	A/A	93	43.87	88	44.67	1		0.996[a]
miR-27a	A/G	94	44.34	86	43.65	0.98	(0.64-1.49)	0.950
	G/G	25	11.79	23	11.68	1.04	(0.54-1.98)	0.970
	AG + GG *vs* AA					1.01	(0.68-1.51)	0.954
	[G] *vs* [A]					0.999	(0.71-1.39)	0.995
	Trend	212		197		0.99	(0.8-1.22)	0.9118[a]
miR-146a	G/G	124	58.49	115	58.38	1		0.761[a]
	C/G	79	37.26	70	35.53	0.93	(0.61-1.41)	0.740
	C/C	9	4.25	12	6.09	1.31	(0.52-3.27)	0.556
	CG + CC *vs* GG					1.03	(0.69-1.54)	0.879
	[C] *vs* [G]					1.37	(0.56-3.33)	0.494
	Trend	212		197		0.97	(0.79-1.19)	0.7558[a]
miR-196-a2	C/C	87	41.04	82	41.62	1		0.6098[a]
	C/T	103	48.58	89	45.18	0.95	(0.62-1.45)	0.794
	T/T	22	10.38	26	13.2	1.32	(0.69-2.54)	0.415
	CT + TT *vs* CC					1.01	(0.68-1.51)	0.951
	[T] *vs* [C]					1.04	(0.75-1.45)	0.811
	Trend	212		197		1.08	(0.8-1.46)	0.5987[a]

P-values are calculated according to Wald's test. [a]*P*-values according to likelihood ratio-test; [1]Age and sex adjusted; CRC: Colorectal cancer; OR: Odds ratio; CI: Confidence interval.

polymorphism using the χ^2 test in patients and controls separately. Allelic frequencies were estimated by the "counting method" and differences in allele frequencies between case and control subjects were tested using the likelihood ratio χ^2 tests for 2 $\pounds$ 2 tables (two alleles, case *vs* control subjects). The homozygote of the most frequent allele was used as a reference for calculating the OR. For an OR and 95% confidence interval, logistical regression was used based on a model for sex and age of the patients. Data analysis was performed using the Statistica v. 9.0 (Statsoft Inc., Tulsa, OK, United States) program package. Values of $P < 0.05$ were considered statistically significant.

RESULTS

All polymorphisms met the criteria of the Hardy-Weinberg equilibrium in the individual patient and control groups. Logistic regression modeling was used to estimate the odds ratios of the investigated genotypes and alleles of SNPs rs2910164, rs11614913 and rs3746444 in CRC cases as well as in the controls (Table 1). All of the examined polymorphisms displayed a clear lack of statistically significant associations with colorectal cancer risk.

DISCUSSION

Sporadic colorectal cancer is a multifactorial disease with multiple genetic determinants of varied significance. Numerous SNP analyses of sporadic colorectal cancer were conducted in order to clarify the genetic background. It has been hypothesized that polymorphic genetic variants involved in metabolism, DNA repair and apoptosis are linked to susceptibility to colorectal cancer[20]. Although alterations in miRNA function have been detected in a broad spectrum of hematological malignancies and solid tumors[21-23], including CRC[24], only two studies performed to date have focused on miRNA-associated SNPs in CRC; although these studies were carried out in the Chinese population[13,14], their results were contradictory. Although it has been hypothesized that SNPs in miRNA genetic regions may affect the transcription of pri-miRNA transcripts, processing of miRNA precursors to mature miRNAs or miRNA target interactions, genetic variants in pre-miRNA regions are rare and unlikely to be functionally important, mainly due to the serious pressure imposed by natural selection on the evolutionary conserved pre-miRNA sequences[3].

In our study, we performed a case-control study of the three most frequently studied SNPs in miRNA genes (rs11614913 in miR-196-a2, rs895819 in miR-27a and rs2910164 in miR-146a), to investigate the degree of risk of CRC in the Central-European Caucasian population.

As it has been experimentally validated that the rs11614913 polymorphism located in the miR-196-a2 mature sequence affects the maturation and effect of target mRNA possibility, it is biologically plausible that genetic variation of hsa-miR-196a2 could modulate cancer susceptibility. In accordance with this finding, rs11614913 is one of the most frequently studied SNPs associated with miRNAs in case-control studies of a wide range of solid cancers[5-14]. For example, Hu *et al*[8] reported that the CC homozygous genotype of rs11614913 located in miR-196a2 was associated with a statistically significant increase in the mature miR-196a and a worse prognosis in non-small-cell lung cancer (NSCLC), proposing that this SNP could serve as a prognostic marker of NSCLC. Another Chinese study reported a clear association between

CC and CC/CT genotypes of rs11614913 and increased risk of breast cancer (OR 1.23; 95% CI: 1.02-1.48)[4]. When reviewed together, the majority of these studies described significant associations of the rs11614913-C allele with susceptibility and/or poor prognosis of lung cancer[7,8], gastric cancer[9], esophageal cancer[10], hepatocellular carcinoma[11] and head and neck cancer[12]. More recently, two Chinese studies focusing on an association between this SNP and susceptibility to CRC and its progression were performed[13,14].

Although the frequency of CC homozygotes of rs11614913 was higher in CRC patients than in healthy controls (41.62% *vs* 41.04%) in our study, the genotypes carrying the C allele (CT and CC) expressed the opposite trend in frequencies (64.21% in CRC *vs* 65.33% in controls). Moreover, the frequency of the C allele in CRC patients (64.21%) was not significantly lower than in healthy controls (65.33%). Furthermore, no significant association between the miR-196a2 polymorphism and the risk of CRC was observed in our study. These results are in agreement with the findings by Chen *et al*[14]. On the other hand, Zhan's group described the C allele as a risk factor for CRC in the Chinese population. Neither of the Chinese studies reported any associations between the rs11614913 polymorphism and CRC progression, including tumor grade, stage, lymph node and distant metastasis[13,14]. The discrepancy in the potential significance of rs11614913 in CRC reported by the above-mentioned independent studies may be due to different molecular pathogenetic mechanisms as different contributors to cancer or population-specific factors such as the different genetic backgrounds of the studied cohorts.

MiR-27a, in general, is a very important miRNA involved in the development of chemoresistance in solid cancer[15]. This study presents the first case-control investigation of the role of the A/G polymorphism (rs895819) in miR-27a in CRC; however, no significant associations were observed. Although the frequency of AA homozygotes was higher in CRC patients than in healthy controls (44.67% *vs* 43.87%), the frequencies of the genotypes carrying the A allele (AA and AG) did not show significant differences between the study cohorts (66.50% in CRC patients *vs* 66.04% in healthy controls). In gastric cancer, it has been reported that the variant genotypes of rs895819 located at miR-27a conferred a 48% increased risk of developing gastric cancer in the Chinese population; moreover, this trend tended to be age-specific. The authors concluded that elevated levels of miR-27a, through regulating the Zinc finger and BTB domain containing 10 (ZBTB10), result in the over-expression of Sp proteins and Sp-dependent genes, which play important roles in gastric cancer cell survival and angiogenesis[15,25]. Furthermore, Yang *et al*[26] found that the G-allele of rs895819, located in the terminal loop of the pre-miR-27a oncogene, is associated with reduced familial breast cancer risk (OR = 0.88; 95% CI: 0.78-0.99; $P = 0.0287$).

MiRNA-146a and its G to C common polymorphism, rs2910164, located within the sequence or the miR-146a precursor represent another miRNA hotspot evaluated in CRC for the first time in the present study. This SNP leads to change from a G:U pair to a C:U mismatch, and consequently, to reduced levels of pre- and mature miR-146a[17,19]. As BRCA1 and BRCA2, key breast and ovarian cancer susceptibility genes, are predicted targets of miR-146a, the majority of studies have been focused on breast and ovarian cancer. The results of Chen *et al*[16], who primarily studied rs2910164 in breast cancer and postulated that breast and ovarian cancer patients who had at least one variant allele were diagnosed at an earlier age ($P = 0.029$, $P = 0.014$, respectively), were not confirmed by further and larger independent case-control studies performed by Hu *et al*[4] and Catucci *et al*[6]. Garcia *et al*[27] concluded that the rs2910164: G > C SNP in the miR-146a gene is not associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. This case-control study, i.e., the first study to investigate the role of the miR-146a polymorphism, rs2910164, in CRC risk, found no significant association. Although our results did not indicate any significant relationship between the above-mentioned, miRNA-associated, SNPs and risk of CRC, we believe that a more detailed and comprehensive characterization of miRNA SNPs will improve our understanding of the miRNAs involved in CRC onset and progression which is necessary for the development of novel diagnostic and therapeutic strategies and approaches to this deadly disease.

COMMENTS

Background

The Central-European Caucasian population displays an extraordinarily high incidence of sporadic colorectal cancer and although there is a long-term tendency towards a decrease in mortality rates attributed to colorectal cancer, it still represents a major cause of death in the population. Susceptibility to colorectal cancer is typically multifactorial and can be characterized by intensive crosstalk between the genetic background of an individual and environmental factors. Susceptibility genes are involved in metabolic pathways controlled by epigenetic mechanisms where microRNA-associated regulations may play an important role.

Research frontiers

MicroRNAs (miRNAs) are small non-coding RNAs regulating gene expression. It has been recently suggested that single nucleotide polymorphisms (SNPs) in genes encoding mir196-a2, miR-27a and mir146-a may be associated with increased risk of various types of solid cancer. However, no such study of colorectal cancer has been conducted so far. This study investigated three SNPs (rs11614913 in miR-196-a2, rs895819 in hsa-miR-27a and rs2910164 in miR-146a) which were previously reported to be significantly associated with various types of solid cancer.

Innovations and breakthroughs

To the best of our knowledge, this is the first study focusing on the significance of rs11614913 in miR-196-a2, rs895819 in hsa-miR-27 and rs2910164 in miR-146a in sporadic colorectal cancer. The study was conducted using a highly homogenous Central-European Caucasian population with extremely high rates of sporadic colorectal cancer.

Applications

The significance of SNPs in genes encoding miRNAs remains controversial. Positive associations of the investigated polymorphisms were reported in various types of solid cancer. Based on the results, however, the investigated SNPs in miRNA genes do not seem to be major genetic determinants of genetic susceptibility to sporadic colorectal cancer in the Central-European population.

Terminology

Genetic susceptibility refers to inherited predisposition to increased risk of developing a certain disease, typically a multifactorial disease. mRNAs mean short non-coding RNAs, 17-22 nucleotides in length, which regulate gene expression and thereby play significant roles in cancer.

Peer review

The manuscript is well presented and supported by data.

REFERENCES

1 **Bosetti C**, Levi F, Rosato V, Bertuccio P, Lucchini F, Negri E, La Vecchia C. Recent trends in colorectal cancer mortality in Europe. *Int J Cancer* 2011; **129**: 180-191

2 **Center MM**, Jemal A, Ward E. International trends in colorectal cancer incidence rates. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1688-1694

3 **Slaby O**, Bienertova-Vasku J, Svoboda M, Vyzula R. Genetic polymorphisms and microRNAs: new direction in molecular epidemiology of solid cancer. *J Cell Mol Med* 2012; **16**: 8-21

4 **Hu Z**, Liang J, Wang Z, Tian T, Zhou X, Chen J, Miao R, Wang Y, Wang X, Shen H. Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. *Hum Mutat* 2009; **30**: 79-84

5 **Hoffman AE**, Zheng T, Yi C, Leaderer D, Weidhaas J, Slack F, Zhang Y, Paranjape T, Zhu Y. microRNA miR-196a-2 and breast cancer: a genetic and epigenetic association study and functional analysis. *Cancer Res* 2009; **69**: 5970-5977

6 **Catucci I**, Yang R, Verderio P, Pizzamiglio S, Heesen L, Hemminki K, Sutter C, Wappenschmidt B, Dick M, Arnold N, Bugert P, Niederacher D, Meindl A, Schmutzler RK, Bartram CC, Ficarazzi F, Tizzoni L, Zaffaroni D, Manoukian S, Barile M, Pierotti MA, Radice P, Burwinkel B, Peterlongo P. 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-E1057

7 **Tian T**, Shu Y, Chen J, Hu Z, Xu L, Jin G, Liang J, Liu P, Zhou X, Miao R, Ma H, Chen Y, Shen H. A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1183-1187

8 **Hu Z**, Chen J, Tian T, Zhou X, Gu H, Xu L, Zeng Y, Miao R, Jin G, Ma H, Chen Y, Shen H. Genetic variants of miRNA sequences and non-small cell lung cancer survival. *J Clin Invest* 2008; **118**: 2600-2608

9 **Peng S**, Kuang Z, Sheng C, Zhang Y, Xu H, Cheng Q. Association of microRNA-196a-2 gene polymorphism with gastric cancer risk in a Chinese population. *Dig Dis Sci* 2010; **55**: 2288-2293

10 **Ye Y**, Wang KK, Gu J, Yang H, Lin J, Ajani JA, Wu X. Genetic variations in microRNA-related genes are novel susceptibility loci for esophageal cancer risk. *Cancer Prev Res* (*Phila*) 2008; **1**: 460-469

11 **Qi P**, Dou TH, Geng L, Zhou FG, Gu X, Wang H, Gao CF. Association of a variant in MIR 196A2 with susceptibility to hepatocellular carcinoma in male Chinese patients with chronic hepatitis B virus infection. *Hum Immunol* 2010; **71**: 621-626

12 **Christensen BC**, Avissar-Whiting M, Ouellet LG, Butler RA, Nelson HH, McClean MD, Marsit CJ, Kelsey KT. Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. *Clin Cancer Res* 2010; **16**: 3713-3720

13 **Zhan JF**, Chen LH, Chen ZX, Yuan YW, Xie GZ, Sun AM, Liu Y. A functional variant in microRNA-196a2 is associated with susceptibility of colorectal cancer in a Chinese population. *Arch Med Res* 2011; **42**: 144-148

14 **Chen H,** Sun LY, Chen LL, Zheng HQ, Zhang QF. A variant in microRNA-196a2 is not associated with susceptibility to and progression of colorectal cancer in Chinese. *Intern Med J* 2011; Epub ahead of print

15 **Sun Q**, Gu H, Zeng Y, Xia Y, Wang Y, Jing Y, Yang L, Wang B. Hsa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting miR-27a and target gene expression. *Cancer Sci* 2010; **101**: 2241-2247

16 **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**: 1963-1966

17 **Guo H**, Wang K, Xiong G, Hu H, Wang D, Xu X, Guan X, Yang K, Bai Y. A functional varient in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. *Fam Cancer* 2010; **9**: 599-603

18 **Xu T**, Zhu Y, Wei QK, Yuan Y, Zhou F, Ge YY, Yang JR, Su H, Zhuang SM. A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. *Carcinogenesis* 2008; **29**: 2126-2131

19 **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 USA* 2008; **105**: 7269-7274

20 **Naccarati A**, Pardini B, Hemminki K, Vodicka P. Sporadic colorectal cancer and individual susceptibility: a review of the association studies investigating the role of DNA repair genetic polymorphisms. *Mutat Res* 2007; **635**: 118-145

21 **Cortez MA**, Ivan C, Zhou P, Wu X, Ivan M, Calin GA. microRNAs in cancer: from bench to bedside. *Adv Cancer Res* 2010; **108**: 113-157

22 **Sana J**, Hajduch M, Michalek J, Vyzula R, Slaby O. MicroRNAs and glioblastoma: roles in core signalling pathways and potential clinical implications. *J Cell Mol Med* 2011; **15**: 1636-1644

23 **Redova M**, Svoboda M, Slaby O. MicroRNAs and their target gene networks in renal cell carcinoma. *Biochem Biophys Res Commun* 2011; **405**: 153-156

24 **Slaby O**, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. *Mol Cancer* 2009; **8**: 102

25 **Mertens-Talcott SU**, Chintharlapalli S, Li X, Safe S. The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. *Cancer Res* 2007; **67**: 11001-11011

26 **Yang R**, Schlehe B, Hemminki K, Sutter C, Bugert P, Wappenschmidt B, Volkmann J, Varon R, Weber BH, Niederacher D, Arnold N, Meindl A, Bartram CR, Schmutzler RK, Burwinkel B. A genetic variant in the pre-miR-27a oncogene is associated with a reduced familial breast cancer risk. *Breast Cancer Res Treat* 2010; **121**: 693-702

27 **Garcia AI,** Cox DG, Barjhoux L, Verny-Pierre C, Barnes D, Antoniou AC, Stoppa-Lyonnet D, Sinilnikova OM, Mazoyer S. The rs2910164: G > C SNP in the MIR146A gene is not associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Hum Mutat* 2011; Epub ahead of print

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