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BREAST CANCER METASTASIS SUPPRESSOR-1 PROMOTER METHYLATION IN CELL FREE DNA PROVIDES PROGNOSTIC INFORMATION IN NON- Small CELL LUNG CANCER

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BACKGROUND: Breast-cancer metastasis suppressor 1 (BRMS1) gene encodes for a predominantly nuclear protein that differentially regulates the expression of multiple genes, leading to suppression of metastasis without blocking orthotopic tumor growth. The aim of the present study was to evaluate for the first time the prognostic significance of BRMS1 promoter methylation in cell free DNA (cfDNA) circulating in plasma of non-small cell lung cancer (NSCLC) patients. Towards this goal, we examined the methylation status of BRMS1 promoter in NSCLC tissues, matched adjacent non-cancerous tissues and corresponding cfDNA as well as in an independent cohort of patients with advanced NSCLC and healthy individuals.

METHODS: BRMS1 promoter methylation was examined in 57 NSCLC tumors and adjacent non-cancerous tissues, in cfDNA isolated from 48 corresponding plasma samples, in cfDNA isolated from plasma of 74 patients with advanced NSCLC and 24 healthy individuals.

RESULTS: BRMS1 promoter was highly methylated both in operable NSCLC primary tissues (59.6%) and corresponding cfDNA (47.9%) but not in cfDNA from healthy individuals (0%), while it was also highly methylated in cfDNA from advanced NSCLC patients (63.5%). In operable NSCLC, Kaplan-Meier estimates were significantly different in favor of patients with non-methylated BRMS1 promoter in cfDNA, concerning both DFI (p=0.048) and OS (p=0.007). In advanced NSCLC, OS was significantly different in favor of patients with non-methylated BRMS1 promoter in their cfDNA (p=0.003). Multivariate analysis confirmed that BRMS1 promoter methylation has a statistical significant influence both on operable NSCLC patients’ DFI time and OS and on advanced NSCLC patients’ PFS and OS.

CONCLUSIONS: BRMS1 promoter methylation in cfDNA isolated from plasma of NSCLC patients provides important prognostic information and merits to be further evaluated as a circulating tumor biomarker.
THE NFKB1 POLYMORPHISM (RS4648068) IS ASSOCIATED WITH THE CELL PROLIFERATION AND MOTILITY IN GASTRIC CANCER

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BACKGROUND: We have demonstrated previously that NFKB1 single nucleotide polymorphism (SNP) rs4648068 GG homozygote was associated with the increased risk of gastric cancer in Chinese Han population. In this study, we constructed the recombinant plasmid pGL3-GG and pGL3-AA and investigated the function of rs4648068 by cell biology experiments.

METHODS: Quantitative real-time PCR was used to detect NF-KB SNP rs4648068 genotype in the patients with gastric cancer. The section of NFKB1 promoter containing this site were obtained by PCR technique and subcloned into the vector pGL3-Basic. Dual-Luciferase reporter assay was used to detect the transcription activity of the constructed promoter. NFKB1 protein levels were analyzed by western blot after transfection of the recombinant plasmid. Furthermore, proliferation and invasion ability of the transduced cell were also measured and compared.

RESULTS: The transcription activity of rs4648068 (A>G) by dual-Luciferase reporter assay suggested that the luciferase activity of mutation group (pGL3-GG) was greater than that of the control (pGL3-AA), especially at the stimulation of LPS. We found that the luciferase activity was also influenced by pGL3-GG levels. The effects of NFKB1 rs4648068 were enhanced by rs4648065 on the transduced cells. Correspondingly, western blot analysis showed increased levels of p50 protein expression in the mutation groups. Our data indicated that the mutation of SNP rs4648068 strengthened the transcriptional activity of NFKB1 and its expression levels, respectively. In addition, the transduction of pGL3 recombinant plasmid pGL3-GG improved the proliferation and invasion ability of gastric cancer cells.

CONCLUSIONS: The transcriptional activity of NFKB1 was associated with SNP rs4648068, and this functional SNP site has the important effects on cell proliferation and motility.

Keywords: NFKB1, polymorphism, gastric cancer, susceptibility, single nucleotide polymorphism
CIRCULATING MICRORNAS, MIR197, LET7D, MIR150, MIR92, IS INFLUENCED BY SMOKING STATUS IN GENERAL POPULATION

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BACKGROUND: Recently, circulating microRNAs are recognized as biomarkers for human various diseases. It is suggested that circulating miRNAs vary widely according to age, gender, and may also be influenced by life-style factors such as drinking, smoking. However, little is known about the influence of these factors. As a first step for clinical application, we need to reveal association of the serum level of circulating miRNAs to lifestyle factors. We investigated that the association between circulating miRNAs, reported the promising biomarker for lifestyle-related diseases, and smoking status in general population from patient's age > 39, attending a comprehensive health check-up program.

METHODS: This cross-sectional study was a total of 514 residents (men 219 and women 308) attended a health examination in Japan. All subjects provided written, informed consent. The information of daily lifestyle habits was obtained by a questionnaire. Serum levels of circulating miRNAs (miR197, let7d, miR150, miR92, miR103, miR27a, miR195, miR199, miR17, miR130a, miR20a) were quantitated by quantitative real-time PCR. The association of circulating miRNAs with smoking status was evaluated using the odds ratios (ORs) with 95% CIs by logistic regression adjusted for confounders (age, gender, alcohol drinking, smoking, BMI, blood pressure, HbA1c, total cholesterol).

RESULTS: The multivariate-adjusted ORs showed a significant association between the serum levels of circulating miRNAs and smoking status among general population. The OR (95% CI) of the elevated serum miR197 levels was 2.10 (95% CI: 1.10-4.03) among subjects with current smoking status compared to subjects with never smoker. Also, OR of the elevated serum let7d, miR150 and miR92 levels were 3.87 (95% CI: 1.95-7.79), 2.76 (95% CI: 1.44-5.34) and 2.05 (95% CI: 1.44-5.34) among subjects with current smoking status compared to subjects with never-smoking status. There was no significant association of smoking status and other levels of circulating miRNAs.

CONCLUSIONS: There are the association between the serum levels of circulating miRNAs and smoking status in general population. These results are important findings as a first step for clinical application.
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ANALYSIS OF THE CIRCULATING HDL-MIRNA STABILITY FOR PRE-ANALYTICAL CONDITIONS

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BACKGROUND: Recently, several studies showed that serum microRNAs (miRNAs) are stably present in extracellular vesicles, proteins, or lipoproteins. Interestingly, it has been reported that the human HDL-miRNA profile from normal subjects is significantly different than that from familial hypercholesterolemia and coronary artery disease subjects. Thus, HDL-miRNAs hold promise as biomarker for each disease, however, little is known about the preservation stability of HDL-miRNAs. In this study, we investigated the stability of HDL-miRNAs in different storage conditions and the influence of repeated freeze-thaw cycles on the HDL-miRNAs.

METHODS: Highly purified fractions of HDL were prepared from human serum in a four-step protocol: (1) ultracentrifugation method at d=1.006 kg/L to remove the chylomicron and VLDL fractions; (2) polyethylene glycol (PEG) method to remove the LDL fraction; (3) ExoQuick method to remove the exosome; (4) fast-protein liquid chromatography (FPLC) system to remove other proteins. In order to examine the stability of HDL-miRNAs, we purified the HDL fraction from serum immediately after centrifugation of the blood; this sample served as the 0-h control. The HDL fractions were purified from these samples after the remainders of the serum were maintained at room temperature or 4°C for 3-h, 6-h, 12-h, or 24-h and stored at -20°C, or -80°C for 1 week, or 2 weeks. Next, Serum sample was also subjected to 1-5 times freeze-thaw cycles after storage at -20°C. MiR-135a and miR-223 extracted from the HDL fraction were measured with real-time PCR method.

RESULTS: The HDL-miRNAs were found to be stable when maintained at room temperature or 4°C for up to 24-h, or stored at -20°C or -80°C for up to 2 weeks. As for the influence of repeated freeze-thaw cycles, the HDL-miRNAs were also stable at least up to 5 times of freeze-thaw cycles.

CONCLUSIONS: Serum HDL-miRNAs may be stable at various temperatures, and not be affected by repeated freeze-thaw.

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ASSOCIATIONS BETWEEN CIRCULATING MICRORNAs MIR-20A OR MIR-27A AND NON-ALCOHOLIC FATTY LIVER DISEASE IN A GENERAL JAPANESE POPULATION: THE YAKUMO STUDY

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BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) has the potential to become non-alcoholic steatohepatitis (NASH) that develop to fibrosis as well as cirrhosis, hepatocellular cancer. MicroRNAs (miRNAs) are non-coding RNAs and help regulate gene expression at post-transcription levels. Recently, it has been found that miRNAs previously identified in specific tissues can also be detected in various kinds of body fluids, including serum. MiRNA-20a, miR-27a, miR-146a, and miR-320 are known to regulate glucose metabolism and various diseases. However, the role of these circulating miRNAs in NAFLD has never been elucidated. The aim of this study was to investigate the relationship between circulating miRNAs (miR-20a, miR-27a, miR-146, miR-320) levels and NAFLD in subjects attended health examination.

METHODS: A total of 527 residents (men 219 and women 308) attended a health examination in Hokkaido Japan in August 2012. All subjects provided written, informed consent. The presence of intrahepatic steatosis was assessed by ultrasound operated by three registered medical sonographers (Japan Society of Ultrasonics in Medicine). The ultrasonography images were independently reviewed by each sonographer who graded hepatic steatosis normal, mild, or severe. Alcohol consumption was assessed via a questionnaire administered by nursing staff. Serum miRNAs (miR-20a, miR-27a, miR-146, miR-320) were quantitated by quantitative real-time PCR.

RESULTS: Subjects with alcohol consumption ≥30 g/day males or ≥20 g/day in females were excluded from the study, as the definition of NAFLD restricts alcohol consumption. We divided subjects into three groups, normal (n = 383), mild (n = 41) and severe (n = 51). Serum level of miR-20a and miR-27a were more decrease in NAFLD severe group than normal group and NAFLD mild group. There were no significant difference among the three groups in the serum miR-146 and miR-320.

CONCLUSIONS: There were associations between circulating miR-20a or miR-27a and NAFLD in a general Japanese population.