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SHORT COMMUNICATION

A Preliminary Study of the Value of Plasma microRNA-193b and Soluble Urokinase-Type Plasminogen Activator Receptor in Identifying Patients with Early-Stage Colorectal Cancer and Advanced Adenoma

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SUMMARY

Background: **Early detection and management of colorectal cancer (CRC) and colorectal adenoma (CRA) reduces the mortality and morbidity of CRC, but there is a lack of ideal circulation biomarkers.**
Methods: **A total of 80 patients with early-stage CRC and CRA and 30 healthy controls were included in this preliminary study. Plasma samples were collected before colonoscopy and prepared for measurement of microRNA-193b and soluble uPAR.**
Results: **Plasma level of miR-193b was decreased through the normal-adenoma-carcinoma sequence with no significant difference between patients with CRC and advanced CRA. The AUC of ROC curve evaluating the value of miR-193b in discriminating patients with early stage CRC or advanced CRA from patients with non-advanced CRA or normal control subjects was 0.849 (95% CI 0.773 - 0.923, $p < 0.001$). Significant alteration of plasma suPAR is only observed in CRC group ($p < 0.001$).**
Conclusions: **Plasma miR-193b may be a novel candidate biomarker for screening patients with early-stage CRC and advanced CRA.**
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KEY WORDS

miR-193b, soluble uPAR, colorectal cancer, colorectal adenoma

INTRODUCTION

Colorectal cancer (CRC) ranks as the third most common cancer and remains the fourth most common malignant cause of death in the world with considerable health burden [1]. As is well known, a large proportion of CRCs are believed to develop in a stepwise pathway from normal epithelium through premalignant adenomas to malignant adenocarcinomas. Since endoscopic adenoma removal may prevent cancer formation, adequate screening methods to identify advanced colorectal adenoma (CRA) are vital in decreasing the morbidity and mortality of CRC [2].

Compared to colonoscopy, a non-invasive blood-based biomarker test has the advantage in terms of compliance as a widespread screening test since it could be a component of routine check-up tests. However, these screening tests are far from sufficient and a common deficiency of these tests is the low sensitivity for detecting advanced adenomas. For example, in a study of the second-generation methylated SEPT9 test, the sensitivity of this assay for CRC was 74.8%, while the sensitivity for advanced colorectal adenoma (CRA) was only 27.4% [3]. Hence, novel biomarkers for detecting CRC, especially advanced adenoma, are urgently needed in CRC screening.

MicroRNAs (miRNAs) are a class of non-coding RNAs that posttranscriptionally regulate the expression of a wide range of genes implicated in colorectal carcinogenesis by acting as tumor promoters or tumor suppressors. Bartley et al. reported that a total of 230 differentially expressed miRNAs were found in the adenoma-adenocarcinoma sequence, suggesting that microRNAs play important roles in the sequence of molecular events, especially early events [4]. The level of miRNAs in tumor tissue could also be reflected in circulation, and the expression of miRNAs is stably detectable in plasma/serum. Previous studies identified a number of circulating miRNAs in the early detection of CRC, although an obvious deficit in these studies is the lack of adenoma samples. Recently, an increasing number of studies have included patients with CRA [5]. In our previous study, we found that miR-193b is significantly altered in plasma of CRC and CRA patients [6].

In addition, the soluble fraction of urokinase-type plasminogen activator receptor (uPAR), referred to as soluble uPAR (suPAR), is originated from cleavage and release of the membrane-bound uPAR and is found in blood. Recently it has been suggested that suPAR is a potential screening tool for the early detection of HCC in patients with chronic liver disorders [7]. The circulating level of suPAR is significantly elevated in patients with CRC [8]; however, it remains unknown whether the alteration is present in patients with colorectal adenomas.

In this study, the expression of miR-193b and suPAR in plasma of patients with CRC and CRA were preliminarily investigated to explore the potential utility of them as screening biomarkers.

MATERIALS AND METHODS

Selection of subjects and sample collection

Twenty-five patients with early-stage CRC (AJCC stage I - II) confirmed by surgery and fifty-five patients with at least one CRA confirmed by endoscopic removal were included between December 2012 and December 2014 in Department of Gastroenterology, Beijing Shijitan Hospital, Capital Medical University in Beijing, China. Patients with familial adenomatous polyposis, Lynch syndrome, inflammatory bowel disease, or a previous CRC diagnosis were excluded. Adenomatous polyps with > 25% villous component, high-grade dysplasia (HGD), or diameter ≥ 10 mm were considered as advanced CRA [9]. The cases of carcinoma in situ (n = 6) were classified as HGD. In addition, thirty patients with no abnormality in colonoscopy and no other carcinomas revealed were enrolled during the same period as healthy controls. Plasma samples were collected from patients before bowel preparation. This research was reviewed and approved by Institutional Review Board of Beijing Shijitan Hospital, Capital Medical University. The enrolled patients provided written informed consent in accordance with the Declaration of Helsinki.

RNA extraction and miRNA quantification

Total RNA was isolated from plasma using the mirVana miRNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. The optical density of the total RNA extracted was assessed at the wave length 260 and 280 nm on a spectrophotometer to evaluate the concentration and purity. Following RNA extraction, Bulge-Loop™ miRNA qRT-PCR kit (miRQ0002819-1-2, ribobio, Guangzhou, China) was used to perform quantitative real-time PCR (qRT-PCR) according to the manufacturer's instructions. Gene expression levels were evaluated in 96-well arrays for miRNA 193b (miR-193b) (Qiagen, Cat. No. CM1HS0064C) according to manufacturer's instruction as follows: 15 minutes at 95°C, 15 seconds at 95°C, 60 seconds at 60°C for 40 cycles using AB 7500 Fast Real-Time PCR system. Relative gene expression levels were normalized to the internal control gene (5S, MQP-0302) and the fold changes of the target gene expression relative to those of the control group were analyzed by the $2^{-\Delta\Delta CT}$ method.

suPAR measurement

Plasma suPAR concentration (ng/mL) was measured in frozen plasma samples using a Conformité Européene (CE)-approved sandwich ELISA (suPARnostic®, ViroGates A/S, Birkeroed, Denmark) according to the manufacturer's protocol.

Statistics

Statistical analysis was performed using SPSS 17.0 software. The nonparametric two tailed Mann-Whitney *U* test and Kruskal-Wallis test were employed to analyze the expression level of miR-193b and soluble uPAR, Pearson's χ^2 test was used to evaluate the correlation between miR-193b and suPAR. To evaluate the diagnostic value of miR-193b and suPAR, receiver operating characteristics (ROC) curves were depicted. A p-value < 0.05 was considered statistically significant.

Table 1. Characteristics of patients and controls included in this study.

Group	N (male/female)	Age
Cancer	25 (12/13)	60.3 ± 9.5
Advanced adenoma	30 (20/10)	65.0 ± 8.1
Non-advanced adenoma	25 (16/9)	58.4 ± 9.0
Normal	30 (20/10)	55.4 ± 8.6

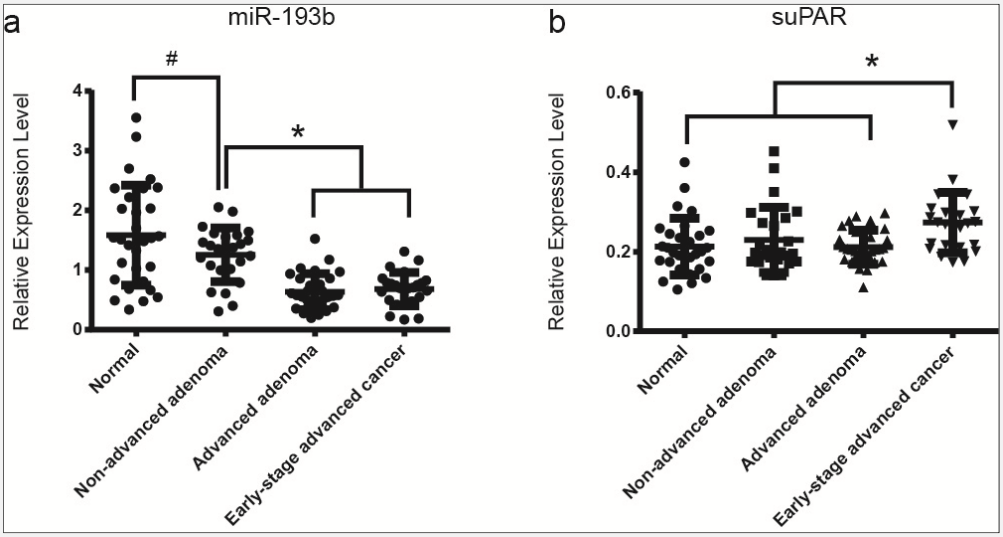

Figure 1. According to the results of PCR, the expression level of miR-193b in plasma is decreased sequentially in normal subjects, patients with non-advanced adenoma and patients with advanced adenoma or early-stage advanced cancer.

However, the level of miR-193b is similar in patients with advanced adenoma and patients with early-stage advanced cancer. The level of suPAR is increased significantly in patients with early-stage advanced cancer; however, there is no significant difference in suPAR expression between normal subjects, patients with non-advanced adenoma, and patients with advanced adenoma. * $p < 0.001$, # $p < 0.05$.

RESULTS

Clinical and pathological features of patients and control subjects are displayed in Table 1. No significant differences were observed for age and gender in four groups. Plasma level of miR-193b was decreased stepwise through the normal-adenoma-carcinoma sequence; however, no significant difference was observed in patients with CRC and patients with advanced CRA (Figure 1a). For suPAR, significant difference was only observed in the CRC group comparing to the other three groups (Figure 1b). ROC curves were depicted to evaluate the value of miR-193b in discriminating patients with early stage CRC or advanced CRA from patients with non-advanced CRA or normal control subjects, and the AUC was 0.849 (95% CI 0.773 - 0.923, $p < 0.001$). If suPAR was combined with miR-193b, the AUC was 0.866 (95% CI 0.796 - 0.935, $p < 0.001$) (Figure 2).

DISCUSSION

Detection of early-stage CRC and advanced CRA is essential in CRC screening, and an effective circulating biomarker is useful for necessitating subsequent colonoscopy and removal of tumors identified. In this preliminary study, the value of two potential biomarkers, miR-193b and suPAR in discriminating CRC and advanced CRA were evaluated.

Mir-193b is an intensively studied microRNA mainly featuring as a tumor suppressor gene. It is reported that

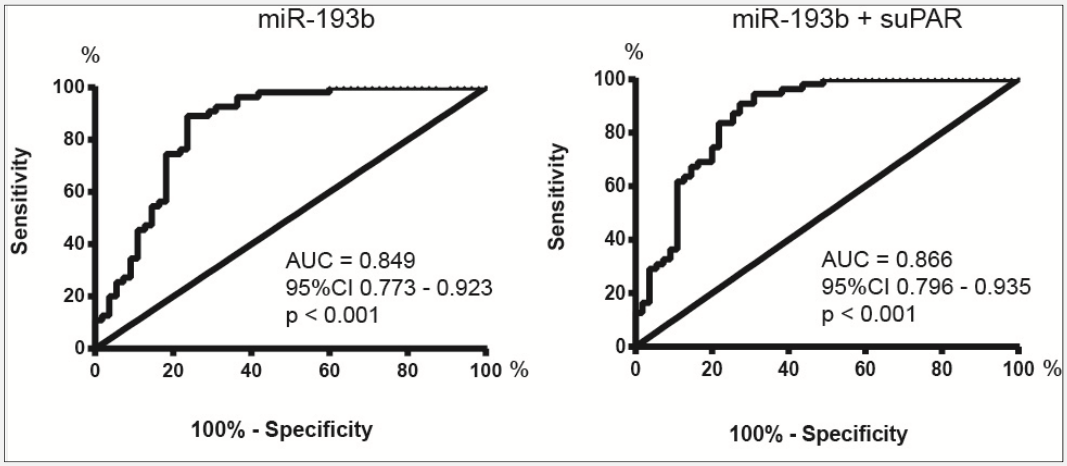

Figure 2. ROC curve analysis of plasma miR-193b level to distinguish advanced adenoma (n = 30) and early-stage cancer (n = 25) from non-advanced adenoma (n = 25) and normal control subjects (n = 30) showed that miR-193b distinguishes patients with early-stage advanced colorectal cancer (CRC) or advanced CRA (n = 55) from patients with non-advanced CRA or healthy controls (n = 55) with AUC = 0.849 (95% CI 0.773 - 0.923, p < 0.001), if suPAR was combined, the AUC was 0.866 (95% CI 0.796 - 0.935, p < 0.001).

miR-193b targets several classical oncogenes, such as cyclin D1, k-ras, STMN1, etc., in multiple malignancies [10-12]. In CRC, it has recently been suggested that miR-193b is also down-regulated significantly and targets stathmin-1 to inhibit the growth and invasion of tumor cells [10], which is consistent with our findings [6]. However, another study suggested that miR-193b exerts an oncogenic effect in CRC [13], suggesting that the role of miR-193b in CRC needs to be determined in future research. The value of circulating miR-193b as a biomarker is currently elusive. Madhavan et al. reported that plasma miR-193b level was associated with survival in breast cancer [14]. However, the significance of circulating miR-193b in CRC and CRA is still unknown.

In our previous study, miR-193b was identified as an obviously altered miRNA in circulation of patients with advanced colorectal neoplasm (including advanced CRA and CRC) using a microRNA array. Hence, in this study, the potential value of miR-193b in identifying CRC and CRA was further explored. Interestingly, we find that miR-193b is indeed down-regulated in adenoma-carcinoma sequence, but the significant alteration is present since the stage of advanced CRA, suggesting that miR-193b is a potential key miRNA in the early stage of colorectal carcinogenesis. Since the level of miR-193b is similar in patients with advanced CRA and CRC, we evaluate the value of miR-193b in distinguishing patients with advanced CRA and CRC from patients with non-advanced CRA and healthy controls using ROC curve, and the result showed that miR-193b is useful for identifying advanced colorectal neoplasm, since the prompt removal of advanced CRA is essential in CRC screening. Hence, we think miR-193b is worthy of being investigated in a large-size study in the future. In addition, suPAR is a soluble fraction of uPAR, which is involved in malignancy progression. Recently suPAR has been suggested to be a low-grade inflammation marker correlating with risk of several cancers in general population [15] and is valuable in early detection of HCC [7]. Simultaneously, uPA, another crucial component of uPA/uPAR system, is reported to be inhibited by miR-193b [16], suggesting a link between miR-193b and uPA/uPAR system. Hence, we also investigated the value of suPAR in advanced colorectal neoplasm. Although the level of plasma suPAR is indeed significantly elevated in CRC, there is no difference between patients with advanced CRA, non-advanced CRA, and healthy controls. It seems that suPAR is not involved in early-stage carcinogenesis of CRC. Due to the limited samples of CRC, we did not investigate the value of suPAR in distinguishing CRC.

In total, in this small-size preliminary study, we propose that miR-193b is a potential biomarker for identifying early-stage advanced colorectal neoplasm (including early-stage CRC and advanced CRA). Future research is warranted to investigate its value.

Declaration of Interest:
No interest declared.

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