A R T I C L E    I N F O

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A B S T R A C T

Early detection and treatment are crucial for increasing the five-year survival rates of various cancers. Low-cost and convenient cancer screening tests are also critically important. Circulating microRNAs are reported as potential biomarkers for various cancers. Recently, miR-1307-3p was found to be a cancer-related microRNA. We evaluated the expression levels of miR-1307-3p in sera obtained from 254 patients with thirteen types of cancer (colon cancer, lung cancer, gastric cancer, liver cancer, bladder cancer, esophageal cancer, breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, biliary tract cancer, brain cancer, sarcoma) and 27 non-cancer samples using isothermal amplification and microRNA chip. The expression levels of miR-1307-3p in sera obtained from cancer patients were clearly different from those obtained from non-cancer samples and differentiated the validation cohort into cancer patients and non-cancer control with high accuracy (AUC: 0.98; sensitivity: 0.98; specificity: 0.85). These results showed the potential relevance of miR-1307-3p in serum for the development of new diagnostic examination tools for cancer patients.

1. Introduction

Cancer is a major health problem in developed nations worldwide [1]. The early detection of cancer leads to the prolongation of the survival rate [2]. However, the detection of early-stage cancer is still difficult. In the case of pancreatic cancer, half of patients are diagnosed as stage IV and the five-year survival rate is only 10% [3]. The screening of early-stage cancers is imperative.

MicroRNAs (miRNAs) are small (18–23 nucleotides), noncoding RNAs that regulate gene expression by binding target messenger RNAs (mRNAs) inside the cell [4]. Recent studies have proved that miRNAs are implicated in cancer [5]. Firstly, the studies of miRNA concerning cancer have focused on tissue samples [6], while circulating miRNAs have received increased attention [7]. MicroRNAs are associated with proteins or extracellular vesicles in the blood and they are protected from nuclease degradation [8] and have potential as less invasive biomarkers of cancer [9, 10, 11, 12].

Recently, hsa-miR-1307-3p has been reported in various cancers. Shimomura et al. reported that miR-1307-3p increased in breast cancer patients’ sera [13] and Han et al. also reported that it stimulates breast cancer expression [14]. Qiu et al. reported that increased expression of miR-1307-3p significantly promotes prostate cancer progression [15].

Chen et al. reported that ovarian cancer cell chemoresistance was promoted by miR-1307-3p [16]. Furthermore, miR-1307-3p was related with chemotherapy of colon cancer [17]. These findings suggest that miR-1307-3p has potential as cancer-related miRNA.

In this study, we demonstrated clear differences in the expression levels of miR-1307-3p in sera of thirteen types of cancer patients and non-cancer samples, suggesting the potential of miR-1307-3p as a diagnostic marker for various cancers.

2. Material and methods

2.1. Materials and reagents

The human sera (non-cancer samples:27, 13 types of cancer patients: 76) used for the experiment were purchased from Kohjin Bio Company, Ltd., and Kishida Chemical Company, Ltd. Sera of cancer patients (13 types of cancer patients:178) were also obtained from National Cancer Center (NCC) Japan between 2017 and 2018 (Table 1). Informed consent was obtained from all patients, and ethics approval for this study was obtained from the ethics committee for human genome/gene analysis research at the Corporate Research & Development Center, Toshiba Corporation. The cancer patients and non-cancer samples were divided into the training (n...
= 90) and validation (n = 189). The miCURY RNA Extraction Kit (Exiqon) was used for miRNAs extraction from serum. The primers used for the reverse transcription (RT), elongation (EL), and isothermal amplification (IA) reactions and synthetic miR-1307-3p were purchased from Life Technologies Japan and FASMAC Co., Ltd (Table 2). qRT-PCR for miR-1307-3p was performed with the TaqMan Advanced miRNA assay kit (Thermo Fisher). Ruthenium hexamine (RuHex) for electrochemical indicator was purchased from Sigma-Aldrich. The electrode substrate, a liquid-channel, and a plastic cassette for microRNA chip were purchased from Toshiba Hokuto Electronics Company, Ltd.

2.2. miRNA extraction and pre-amplification

The miRNAs extracted from 300 μL serum samples using commercially available kit was eluted into 30 μL of RNA storage solution (Thermo Fisher). The principle underlying the pre-amplification of miRNAs based on RT and EL reactions was reported in previous paper [18].

2.3. Preparation of an electrochemical microRNA chip

The forward primer and backward primer (32 μM each) and loop primer (16 μM) used for IA were dissolved in 10 mM Tris-HCl (pH 8.0) containing 0.003% xylene cyanol, after which 100 nL was spotted on the surface of the liquid-flow channel by using AD1620 Aspirate/Dispense Platform (BioDot) and dried at room temperature. After fixing the primers by adsorption, the assembled microRNA chip cassette was vacuum sealed and stored at -20 °C before use.

2.4. Quantitative real-time electrochemical measurement of the isothermal amplification reaction

The real-time electrochemical isothermal amplification used in this study was based on the previous work [18, 19, 20]. The positively charged RuHex was associated with negatively charged ampiclon in the amplification solution and the cathodic peak current (Ipc) derived from RuHex was dramatically decreased according to amplification reaction (Figure 1). Following the progress of amplification reaction, the coproduced and negatively charged pyrophosphate was increased in the solution, and coprecipitation of RuHex associated with both ampiclon and pyrophosphate causes an increase in the cathodic peak current. In this study, we used the former change of signal instead of the later change of signal reported in previous study [18]. The amplification time for the microRNA (Ti) was defined as the time until the signal crossed a threshold of -2 (d (Ipc)/dt).

2.5. Statistical analysis

In the present study, expression level of miR-1307-3p was compared between 13 types of cancer and non-cancer samples. JMP8 (SAS Institute Inc., NC, USA) was used to analyzed and display the data. A receiver operating characteristic (ROC) curve analysis was performed, and the area under the ROC curve (AUC) was calculated to evaluate the diagnostic value. The optimal cut-off value, sensitivity, and specificity were determined by calculating the Youden index. P < 0.05 was considered statistically significant.

3. Results

3.1. Characteristic of patients enrolled in this study

The clinical characteristics of the patients enrolled in this study are shown in Table 1. Twenty-seven non-cancer control subjects were randomly divided into training cohort (14) and validation cohort (13). The training cohort included commercially available samples from 76 patients with 13 types of cancer (biliary tract cancer, bladder cancer,
assay ($n = 13$). An analysis of the data revealed a relatively good correlation between the results of the microRNA chip electrochemical measurements and the qRT-PCR assay ($R^2: 0.74$) (Figure 3). These results indicate that the microRNA chip serves convenient and real-time quantification of miRNA extracted from serum. In the experiment of training and validation cohort, two standard points ($5 \times 10^2$ copies/μL and $5 \times 10^5$ copies/μL) were used for calibration of quantitative analysis of miR-1307-3p.

3.3. Comparison of 13 types of cancers with non-cancer control in training cohort

In the training step, the expression levels of miR-1307-3p in serum were estimated in a small set of cancer ($n = 76$) and non-cancer control ($n = 14$). The expression levels were significantly increased in cancer compared with non-cancer control ($p < 0.0001$) (Figure 4A, B). To apply these findings to clinical and practical settings, logistic regression

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**Figure 1.** Scamatic diagram of microRNA detection using electrochemical microRNA chip. (1): Isothermal amplification reaction in microRNA chip. (2): Electrochemical reaction of RuHex in amplification solution. RuHex is uniformly distributed in solution in early stage of amplification reaction (A). The positively charged RuHex is associated with negatively charged amplicon produced in amplification solution and the cathodic peak current ($I_{PC}$) derived from RuHex is dramatically decreased (B). Following the progress of amplification reaction, the coproduced pyrophosphate is increased in the solution, and coprecipitation of RuHex associated with both amplicon and pyrophosphate causes an increase in the cathodic peak current (C).

**Figure 2.** Calibration curve of quantification analysis of miR-1307-3p using microRNA chip ($n = 3$).

**Figure 3.** Correlation plots for quantification results of the miR-1307-3p determined by microRNA chip and qRT-PCR ($n = 14$). non-cancer control (closed circle), breast cancer with stage II (open triangle), breast cancer with stage III (open square).
analysis of cancer patients and non-cancer control was performed using the individual profile of the miR-1307-3p. The cut-off value was determined at 3.66 from the ROC curve (Figure 5A). The expression level of miR-1307-3p in serum could distinguish cancer and non-cancer control with a sensitivity of 88%, a specificity of 100% and AUC of 0.99.

Figure 6A showed the expression level of miR-1307-3p according to clinical stages in 13 types of cancers. The AUC value of stage I, II, III and IV were 0.98, 0.99, 0.99 and 0.93, respectively. The expression level was not depending on the stages of cancers.

3.4. Comparison of 13 types of cancers with non-cancer control in validation cohort

In the validation step, a total of 191 serum samples were examined to evaluate the diagnostic potential of miR-1307-3p. The expression levels of miR-1307-3p in serum showed a significant increase in cancer patients ($p < 0.0001$) compared with non-cancer control (Figure 4C, D). The sample diagnosed as stage 0 was included in the validation cohort. ROC analyses showed that the expression levels of miR-1307-3p were able to discriminate cancer patients from non-cancer control with AUC value of 0.98 (Figure 5B). The sensitivity, specificity, PPV, and NPV are shown in Table 3. The results demonstrated the high sensitivity, specificity, PPV, and NPV of the expression levels for predicting cancer patients and non-cancer control in the training and validation cohorts. The sample diagnosed as stage 0 was included in the validation cohort. The AUC value of stage 0, I, II, III and IV were 1.00, 0.98, 0.98, 0.96 and 0.89, respectively (Figure 6B).

4. Discussion

The occurrence of cancer is influenced by genetic and environmental factors. Early diagnosis and early treatment are the most effective methods to improve prognosis. Although detection of tumor markers such as PSA, AFP, and CEA can provide references for diagnosis and is of great importance in the follow-up of cancers, there is lack of positive clinical evidence in early diagnosis [21, 22]. Therefore, searching for
sensitive and specific early screening markers is important for early diagnosis of cancer.

In this study, we evaluated the expression level of miR-1307-3p in sera obtained from cancer patients and non-cancer control using microRNA chip. MiR-1307-3p could be useful for the screening of patients with cancer. The sensitivity, specificity, PPV (%), and NPV (%) of the profile were tolerable for the prediction of cancer patients and non-cancer control in the training and validation cohorts. There is a possibility of discriminating the early stages of cancer, such as stage 0 and stage I, from non-cancer control.

There was no correlation between the expression levels of miR-1307-3p and clinical stages in our study. Shimomura also reported the combination of serum miRNAs for detecting breast cancer in early stage using microarray and each miRNA expression level used to compose the diagnostic index showed no correlation with the clinical stages [13]. MiR-1307-3p was reported as both an oncogenic [13, 14] and an anti-oncogenic [23] miRNA. It is supposed that the action of miR-1307-3p with respect to cancer is dependent on the type of cancer. However, the function and mechanism of miR-1307-3p with respect to cancer have yet to be clarified. It is necessary to investigate various cell species in order to analyze the exact function.

In conclusion, we demonstrated a miR-1307-3p profile in sera from cancer patients compared to non-cancer control, and showed the potential diagnostic values of these differences for clinical usage. MiR-1307-3p was associated with 13 types of cancer and may therefore serve as a novel screening biomarker for cancer.

Table 3. Discriminant analysis between 13 types of cancer and non-cancer control in training and validation sets.

| Tumor Type            | Stage | Sensitivity | Specificity | PPV   | NPV   | AUC   |
|-----------------------|-------|-------------|-------------|-------|-------|-------|
| Biliary tract cancer  |       | 0.83 (4/5)  | 1.00 (14/14) | 1.00  | 0.93  | 0.93  |
|                       | validation | 1.00 (6/6)  | 0.85 (11/13)  | 0.75  | 1.00  | 1.00  |
| Bladder cancer        |       | 0.60 (3/5)  | 1.00 (14/14)  | 1.00  | 0.88  | 0.88  |
|                       | validation | 1.00 (2/2)  | 0.85 (11/13)  | 0.50  | 1.00  | 1.00  |
| Brain cancer          |       | 0.80 (4/5)  | 1.00 (14/14)  | 1.00  | 0.93  | 0.93  |
|                       | validation | 1.00 (20/20) | 0.85 (11/13)  | 0.91  | 1.00  | 1.00  |
| Breast cancer         |       | 0.86 (12/14) | 1.00 (14/14)  | 1.00  | 0.88  | 0.88  |
|                       | validation | 0.95 (20/21) | 0.85 (11/13)  | 0.91  | 0.92  | 0.92  |
| Colon cancer          |       | 1.00 (6/6)  | 1.00 (14/14)  | 1.00  | 1.00  | 1.00  |
|                       | validation | 1.00 (17/17) | 0.85 (11/13)  | 0.90  | 1.00  | 1.00  |
| Esophageal cancer     |       | 1.00 (5/5)  | 1.00 (14/14)  | 1.00  | 1.00  | 1.00  |
|                       | validation | 1.00 (13/13) | 0.85 (11/13)  | 0.87  | 1.00  | 1.00  |
| Gastric cancer        |       | 1.00 (6/6)  | 1.00 (14/14)  | 1.00  | 1.00  | 1.00  |
|                       | validation | 1.00 (18/18) | 0.85 (11/13)  | 0.90  | 1.00  | 1.00  |
| Liver cancer          |       | 1.00 (5/5)  | 1.00 (14/14)  | 1.00  | 1.00  | 1.00  |
|                       | validation | 0.67 (2/3)  | 0.85 (11/13)  | 0.50  | 0.92  | 0.92  |

(continued on next page)
Table 3 (continued)

| cancer            | training sensitivity | training specificity | training PPV | training NPV | training AUC |
|-------------------|----------------------|----------------------|--------------|--------------|--------------|
| lung cancer       |                      |                      |              |              |              |
|                   | 1.00 (3/3)           | 1.00 (14/14)         | 1.00 (3/3)   | 1.00 (14/14) |              |
| validation        | 0.95 (18/19)         | 0.85 (11/13)         | 0.90 (18/20) | 0.92 (11/12) |              |
| ovarian cancer    |                      |                      |              |              |              |
|                   | 1.00 (6/6)           | 1.00 (14/14)         | 1.00 (6/6)   | 1.00 (14/14) |              |
| validation        | 1.00 (7/7)           | 0.85 (11/13)         | 0.78 (7/9)   | 1.00 (11/11) |              |
| pancreatic cancer |                      |                      |              |              |              |
|                   | 0.83 (5/6)           | 1.00 (14/14)         | 0.93 (5/5)   | 1.00 (14/15) |              |
| validation        | 1.00 (18/18)         | 0.85 (11/13)         | 0.90 (18/20) | 1.00 (11/11) |              |
| prostate cancer   |                      |                      |              |              |              |
|                   | 0.80 (4/5)           | 1.00 (14/14)         | 1.00 (4/4)   | 0.93 (14/15) |              |
| validation        | 1.00 (25/25)         | 0.85 (11/13)         | 0.93 (25/27) | 1.00 (11/11) |              |
| Sarcoma           |                      |                      |              |              |              |
|                   | 0.80 (4/5)           | 1.00 (14/14)         | 1.00 (4/4)   | 0.93 (14/15) |              |
| validation        | 1.00 (9/9)           | 0.85 (11/13)         | 0.82 (9/11)  | 1.00 (11/11) |              |
| all cancers       |                      |                      |              |              |              |
|                   | 0.88 (67/76)         | 1.00 (14/14)         | 0.99 (67/67) | 0.61 (14/23) | 0.99         |
| validation        | 0.98 (175/178)       | 0.85 (11/13)         | 0.99 (175/177)| 0.79 (11/14) | 0.98         |

Declarations

Author contribution statement

Koji Hashimoto: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mika Inada: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Yusuke Yamamoto: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Takahiro Ochiya: Conceived and designed the experiments; Analyzed and interpreted the data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare that there is no conflict of interest.

Additional information

No additional information is available for this paper.

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