Heliyon 7 (2021) e07919

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Preliminary evaluation of miR-1307-3p in human serum for detection of 13 types of solid cancer using microRNA chip

Koji Hashimoto [a,*], Mika Inada [a], Yusuke Yamamoto [b], Takahiro Ochiya [c]

[a] Corporate Research & Development Center, Toshiba Corporation, Kawasaki, Japan
[b] Division of Molecular and Cellular Medicine, National Cancer Center Research Institute Japan, Tokyo, Japan
[c] Department of Molecular and Cellular Medicine, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan

ARTICLE INFO

Keywords:
MicroRNA chip
Serum
Liquid biopsy
Isothermal amplification

ABSTRACT

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

* Corresponding author.
E-mail address: kouji.hashimoto@toshiba.co.jp (K. Hashimoto).

https://doi.org/10.1016/j.heliyon.2021.e07919
Received 17 January 2021; Received in revised form 4 April 2021; Accepted 31 August 2021

= 90) and validation (n = 189). The miRCURY 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-flow 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 amplicon 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 amplicon 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, brain cancer, breast cancer, colon cancer, esophageal cancer, gastric cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma). The validation cohort included 178 patients sample with 13 types of cancer obtained from NCC. There were no statistical differences in age and gender between the cancer patients and non-cancer control in the training and validation cohorts.

Table 1. Characteristic of cancer patients and non-cancer control enrolled in this study.

	Training	Validation	p-value*
Cancer patient			
Age (year)	57.9 ± 12.9	59.5 ± 10.4	0.33
Gender			
Male	30	95	0.06
Female	46	83	
Cancer types			
biliary tract cancer	5	6	0.03
bladder cancer	5	2	
brain cancer	5	20	
breast cancer	14	21	
colon cancer	6	17	
esophageal cancer	5	13	
gastric cancer	6	18	
liver cancer	5	3	
lung cancer	3	19	
ovarian cancer	6	7	
pancreatic cancer	6	18	
prostate cancer	5	25	
sarcoma	5	9	
Stage			
0	0	2	<0.001
I	14	33	
II	31	13	
III	23	13	
IV	3	2	
unknown	5	115	
Non-cancer control			
Age (year)	53.8 ± 13.3	55.2 ± 11.5	0.78
Gender			
Male	8	4	0.32
Female	6	9	

*p-value was determined using Pearson's Chi-squared test with Yates' continuity correction.

3.2. Quantitative analysis of miR-1307-3p using microRNA chip

Figure 2 shows the calibration curve of miR-1307-3p using microRNA chip. MiR-1307-3p was also quantified by a TaqMan-based qRT-PCR

Table 2. Sequences of oligonucleotides used in this work.

		Sequence (5′→3′)
miR-1307-3p		ACUCGGCGUGGCGUCGGUCGUG
RT primer		CCAGTCCGCCACTGTACTCACGACC
EL primer		AGGGTTGGAGGTTCATGTCAAGGCCCA GTCTCACGTATTCCACTGACCACCAGTCGCACTGA GGGGACTCGGCGTGGCGT
IA	Forward primer	TGGAATACGTGAGACTGGGC CTTTTTTTTTAGGGTTGGAGGTTCATGTCA
	Backward primer	CTGACCACCAGTCGCACTGAGCCAGTCCGCCACTGTACT
	Loop primer	TGGCGTCGGTC

RT: reverse transcription, EL: elongation, IA: isothermal amplification.

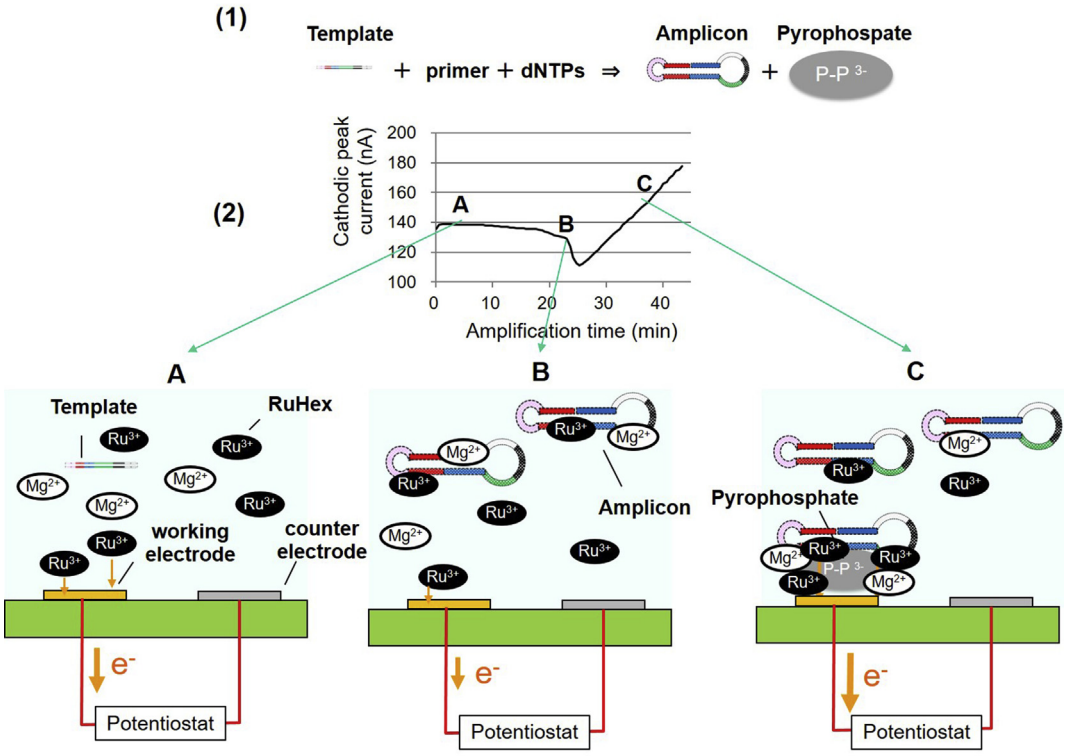

Figure 1. Scamatic diagram of microRNA detection using electrochemical microRNA chip. (1): Isothermal amplification reaction in microRNA chip. (2): Electrochemical reaction of RuHex in amplification solution. RuHex is uniformly distributed in solution in early stage of amplification reaction (A). The positively charged RuHex is associated with negatively charged amplicon produced in amplification solution and the cathodic peak current (Ipc) 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).

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 (5×10^2 copies/μL and 5×10^5 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

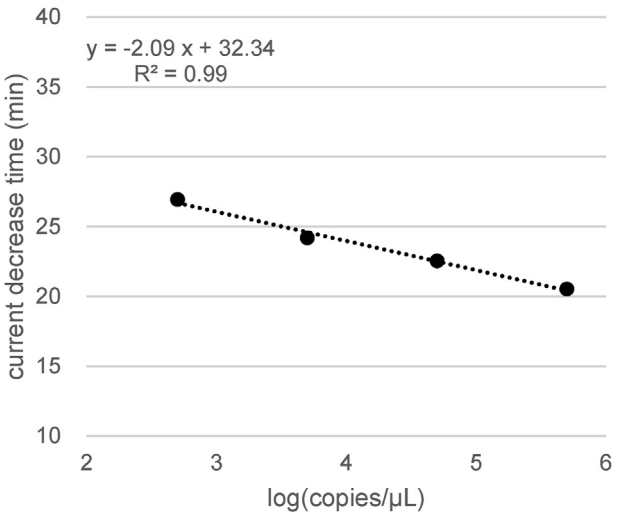

Figure 2. Calibration curve of quantification analysis of miR-1307-3p using microRNA chip (n = 3).

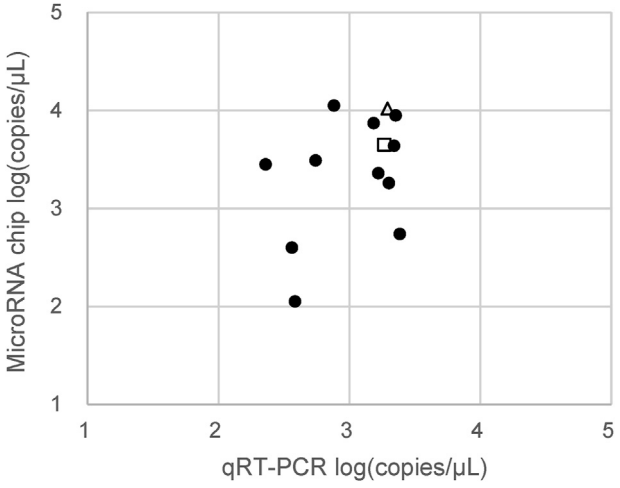

Figure 3. Correlation plots for quantification results of the miR-1307-3p determined by microRNA chip and qRT-PCR (n = 14). non-cancer control (closed circle), breast cancer with stage II (open triangle), breast cancer with stage III (open square).

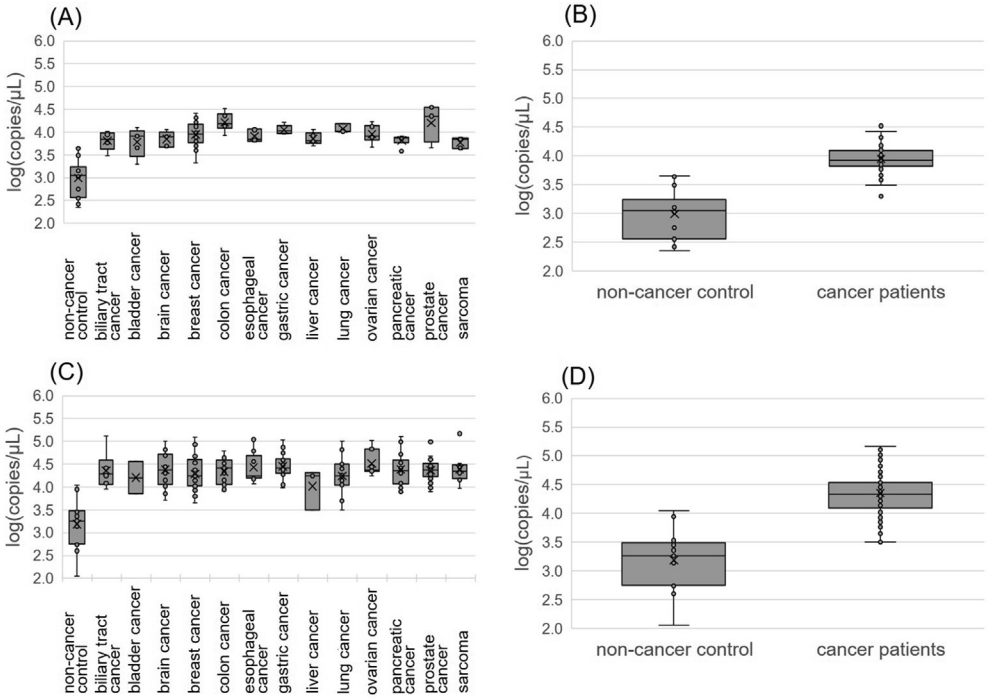

Figure 4. Expression level of miR-1307-3p of 13 types of cancer patients and non-cancer control in training and validation set. (A) The expression level of miR-1307-3p was compared with each of 13 types of cancers and non-cancer control in training set. (B) The expression level of miR-1307-3p was compared with all cancers and non-cancer control in training set. (C) The expression level of miR-1307-3p was compared with each of 13 types of cancers and non-cancer control in validation set. (D) The expression level of miR-1307-3p was compared with all cancers and non-cancer control in validation set.

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 is 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

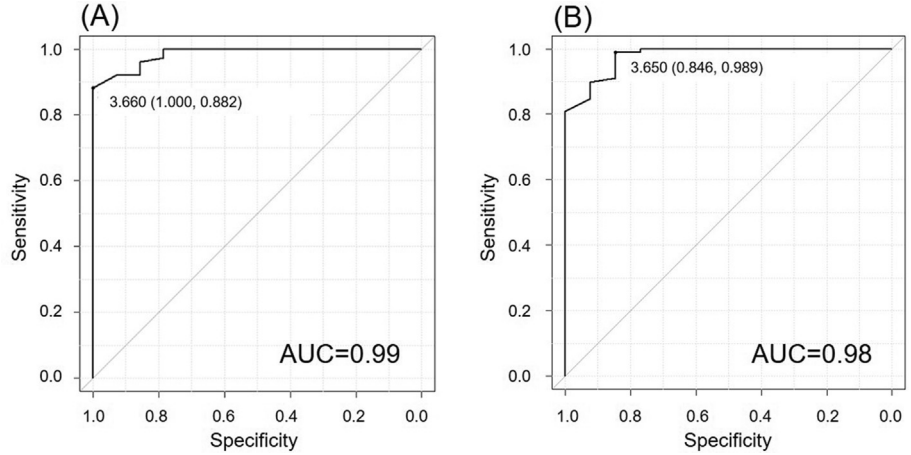

Figure 5. ROC curve analysis for the prediction of 13 types of cancer using miR-1307-3p (A) in training set and (B) in validation set.

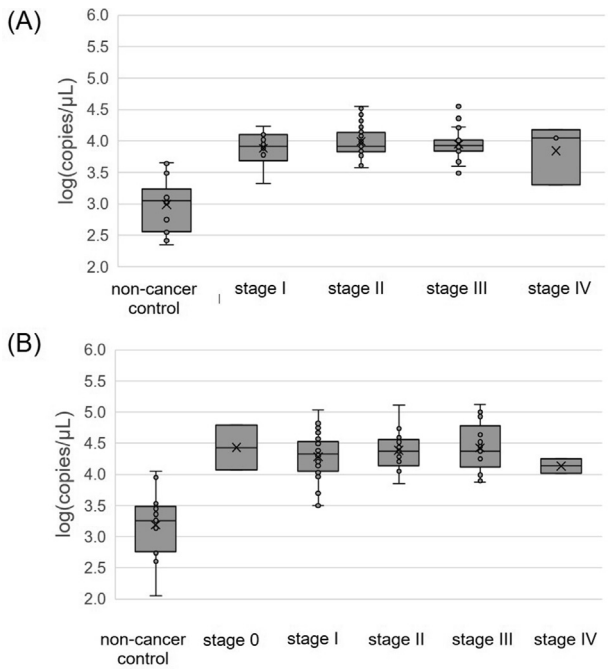

Figure 6. Expression level of miR-1307-3p according to clinical stages (A) in training set and (B) in validation set.

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 on 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.

		sensitivity	specificity	PPV	NPV	AUC
biliary tract cancer	training	0.83 (4/5)	1.00 (14/14)	1.00 (4/4)	0.93 (14/15)	
	validation	1.00 (6/6)	0.85 (11/13)	0.75 (6/8)	1.00 (11/11)	
bladder cancer	training	0.60 (3/5)	1.00 (14/14)	1.00 (3/3)	0.88 (14/16)	
	validation	1.00 (2/2)	0.85 (11/13)	0.50 (2/4)	1.00 (11/11)	
brain cancer	training	0.80 (4/5)	1.00 (14/14)	1.00 (4/4)	0.93 (14/15)	
	validation	1.00 (20/20)	0.85 (11/13)	0.91 (20/22)	1.00 (11/11)	
breast cancer	training	0.86 (12/14)	1.00 (14/14)	1.00 (12/12)	0.88 (14/16)	
	validation	0.95 (20/21)	0.85 (11/13)	0.91 (20/22)	0.92 (11/12)	
colon cancer	training	1.00 (6/6)	1.00 (14/14)	1.00 (6/6)	1.00 (14/14)	
	validation	1.00 (17/17)	0.85 (11/13)	0.90 (17/19)	1.00 (11/11)	
esophageal cancer	training	1.00 (5/5)	1.00 (14/14)	1.00 (5/5)	1.00 (14/14)	
	validation	1.00 (13/13)	0.85 (11/13)	0.87 (13/15)	1.00 (11/11)	
gastric cancer	training	1.00 (6/6)	1.00 (14/14)	1.00 (6/6)	1.00 (14/14)	
	validation	1.00 (18/18)	0.85 (11/13)	0.90 (18/20)	1.00 (11/11)	
liver cancer	training	1.00 (5/5)	1.00 (14/14)	1.00 (5/5)	1.00 (14/14)	
	validation	0.67 (2/3)	0.85 (11/13)	0.50 (2/4)	0.92 (11/12)	

(*continued on next page*)

Table 3 (*continued*)

		sensitivity	specificity	PPV	NPV	AUC
lung cancer	training	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	training	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	training	0.83 (5/6)	1.00 (14/14)	1.00 (5/5)	0.93 (14/15)	
	validation	1.00 (18/18)	0.85 (11/13)	0.90 (18/20)	1.00 (11/11)	
prostate cancer	training	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	training	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	training	0.88 (67/76)	1.00 (14/14)	1.00 (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.

Funding statement

This work was supported by Japan Agency for Medical Research and Development (AMED) under Grant Number JP18ae0101014.

Data availability statement

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

Declaration of interests statement

The authors declare the following conflict of interests: Koji Hashimoto and Mika Inada are employees of Toshiba Corporation and the technologies reported in this paper are patent pending. Yusuke Yamamoto and Takahiro Ochiya received research funding from Toshiba Corporation.

Additional information

No additional information is available for this paper.

References

[1] C. Mattiuzzi, G. Lippi, Current cancer epidemiology, J. Epidemiol. Glob. Health 9 (2019) 217–222.
[2] R. Etzioni, N. Urban, Ramsey Scott, et al., The case for early detection, Nat. Rev. Cancer 3 (2003) 243–252.
[3] J.D. Mizrahi, R. Surana, J.W. Valle, et al., Pancreatic cancer, Lancet 395 (2020) 2008–2020.
[4] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[5] C.L. Bartels, G.J. Tsongalis, MicroRNAs: novel biomarkers for human cancer, Clin. Chem. 55 (2009) 623–631.
[6] J. Lu, G. Getz, E.A. Miska, et al., MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.
[7] J. Matsuzaki, T. Ochiya, Circulating microRNAs and extracellular vesicles as potential cancer biomarkers: a systematic review, Int. J. Clin. Oncol. 22 (2017) 413–420.
[8] P.S. Mitchell, R.K. Parkin, E.M. Kroh, et al., Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 10513–10518.
[9] A. Yokoi, J. Matsuzaki, Y. Yamamoto, et al., Integrated extracellular microRNA profiling for ovarian cancer screening, Nat. Commun. 9 (2018).
[10] N. Asano, J. Matsuzaki, M. Ichikawa, et al., A serum microRNA classifier for the diagnosis of sarcomas of various histological subtypes, Nat. Commun. 10 (2019), 1299.
[11] D. Shigemizu, S. Akiyama, Y. Asanomi, et al., Risk prediction models for dementia constructed by supervised principal component analysis using miRNA expression data, Commun. Biol. 2 (2019), 77.
[12] D. Charytonowicz, H. Aubrey, C. Bell, et al., Cost analysis of noninvasive blood-based microRNA testing versus CT Scans for follow-up in patients with testicular germ-cell Tumors, Clin. Genitourin. Cancer 17 (2019) e733–e744.
[13] A. Shimomura, S. Shiino, J. Kawauchi, et al., Novel combination of serum microRNA for detecting breast cancer in the early stage, Cancer Sci. 107 (2016) 326–334.
[14] S. Han, H. Zou, J.-W. Lee, et al., miR-1307-3p stimulates breast cancer development and progression by targeting SMYD4, J. Cancer 10 (2019) 441–448.
[15] X. Qiu, Y. Dou, miR-1307 promotes the proliferation of prostate cancer by targeting FOXO3A, Biomed. Pharmacother. 88 (2017) 430–435.
[16] W.T. Chen, Y.J. Yang, Z.D. Zhang, et al., MiR-1307 promotes ovarian cancer cell chemoresistance by targeting the ING5 expression, J. Ovarian Res. 10 (2017).
[17] Q. Chen, Y. Mao, F. Meng, et al., Rs7911488 modified the efficacy of capecitabine-based therapy in colon cancer through altering miR-1307-3p and TYMS expression, Oncotarget 8 (2017) 74312–74319.
[18] K. Hashimoto, M. Inada, K. Ito, Multiplex real-time loop-mediated isothermal amplification using an electrochemical DNA chip consisting single liquid flow channel, Anal. Chem. 91 (2019) 3227–3232.
[19] K. Hashimoto, M. Inada, K. Ito, Preliminary evaluation for a novel voltammetric analysis of targeted nucleic acid by combining electrochemical DNA chip and digital loop-mediated isothermal amplification, J. Electroanal. Chem. 807 (2017) 104–107.

[20] K. Hashimoto, M. Inada, K. Ito, A novel voltammetric approach for real-time electrochemical detection of targeted nucleic acid sequences using LAMP, Anal. Biochem. 539 (2017) 113–117.
[21] F. Feng, Y. Tian, G. Xu, et al., Diagnostic and prognostic value of CEA, CA19–9, AFP and CA125 for early gastric cancer, BMC Cancer 17 (2017), 737.
[22] D. Ilic, M. Djulbegovic, J.H. Jung, et al., Prostate cancer screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis, BMJ 362 (2018), k3519.
[23] Y. Zheng, Y. Zheng, W. Lei, et al., miR-1307-3p overexpression inhibits cell proliferation and promotes cell apoptosis by targeting ISM1 in colon cancer, Mol. Cell. Probes 48 (2019), 101445.