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Multiplexed microRNA Expression Profiling by Combined Asymmetric PCR and Label-Free Detection using Silicon Photonic Sensor Arrays

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Abstract

Analysis methods based upon the quantitative, real-time polymerase chain reaction are extremely powerful; however, they face intrinsic limitations in terms of target multiplexing. In contrast, silicon photonic microring resonators represent a modularly multiplexable sensor array technology that is well-suited to the analysis of targeted biomarker panels. In this manuscript we employ an asymmetric polymerase chain reaction approach to selectively amplify copies of cDNAs generated from targeted miRNAs before multiplexed, label-free quantitation through hybridization to microring resonator arrays pre-functionalized with capture sequences. This method, which shows applicability to low input amounts and a large dynamic range, was demonstrated for the simultaneous detection of eight microRNA targets from twenty primary brain tumor samples with expression profiles in good agreement with literature precedent.

Introduction

It is well accepted that multiplexed diagnostics can provide a more holistic view of biomolecular dynamics that supports an improved understanding of disease onset and progression.^{1–2} One of the important potential outcomes of multiplexed analysis is a deeper understanding of the role of microRNA (miRNA) molecules and the interconnected networks through which they help regulate protein expression.³ Dysregulation of miRNA levels leads to altered protein expression, which has profound implications in a wide range

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of pathophysiological conditions.^{4–8} An increasing appreciation for plasticity and redundancy within miRNA regulatory networks motivates the development of methods to perform multiplexed measurements to simultaneously profile multiple miRNA expression levels from sample-limited clinical specimens.^{9–10}

Unfortunately, technological gaps exist that have hindered the translation of miRNA-based diagnostics to the clinic.^{10–11} Specifically, RT-qPCR methods, which are very sensitive, relatively rapid, and cost effective, (typically) only measure levels of one miRNA per assay. Microarrays, are well-suited for multiplexed analyses, but are typically slow, less sensitive, and more expensive. Next-generation sequencing allows global profiling and discovery of RNA signatures, including miRNAs; however, it requires complex processing steps and intensive bioinformatics to interpret sequencing reads. As a result, sequencing has yet to emerge as a standard diagnostic tool to guide clinical intervention. That said, previous efforts of sequencing and bioinformatic data reduction have identified panels of miRNAs that are promising as sets of actionable biomarkers,¹² motivating the development of moderately multiplexable miRNA analysis technologies that can fill the present void by providing targeted detection of clinically relevant panels.

Silicon photonic microring resonators are surface-sensitive optical sensors that are intrinsically multiplexable on account of their fabrication via established semiconductor methods. These devices support optical resonances that are sensitive to local environment, and, when functionalized with appropriate capture agents, analyte binding-induced changes in local refractive index can be detected as shifts in resonance wavelength, as previously described.¹³ The sensors can be functionalized with different classes of receptors to afford selectivity towards different classes of biomolecular targets.^{14–19} Applied to the detection of miRNAs, these sensors have been operated in a label-free format,²⁰ as well in both antibody-,²¹ and enzyme-enhanced assays.²² While these studies demonstrated robust performance and multiplexing capacity, they suffered from insufficient limits of detection that required either large sample input or lengthy analysis times, both of which are restrictive for clinical miRNA profiling applications.

In this manuscript we exploit the advantages of both PCR (target-specific amplification) and microring resonators (multiplexed detection) to profile multiple miRNAs from clinically-relevant samples using a label-free, hybridization-based method. While others have successfully coupled solid phase PCR with optical biosensors,²³ we elected to move away from traditional PCR since it produces double stranded DNA products which are not amenable for hybridization based assays. Instead, asymmetric PCR (aPCR) was used to selectively produce single stranded DNA products by using an excess of one of the PCR primers.^{24–25} This combination greatly improves upon previous microring resonator-based miRNA detection assays and allows analyses from only nanograms of total RNA—an amount comparable with standard, single-plex PCR methods. aPCR was originally demonstrated for solution-phase DNA detection²⁴ and has also been combined with surface plasmon resonance (SPR) for detection.²⁶ For RNA analysis, aPCR has been applied to mRNAs using microarrays²⁷ and 16S rRNAs via a magnetic bead-based method.²⁸ Here, we utilize silicon photonic sensor arrays and report a new stem loop primer motif that firstly allows detection of miRNAs using a modified aPCR approach. We then demonstrate the

ability to profile expression levels of eight miRNAs simultaneously from primary surgical glioma specimens.

Experimental

Materials

All nucleic acid sequences were purchased from Integrated DNA Technologies (IDT; Coralville, IA) and are listed in Table S1. The TaqMan® microRNA Reverse Transcription Kit and the Platinum® Multiplex PCR Master Mix were purchased from Thermo Fisher. All buffers and dilutions were prepared in nuclease-free Ultrapure distilled water (Invitrogen). Phosphate-buffered saline (PBS) was obtained from Lonza and was used in the reconstitution of the DNA capture probes. For the functionalization of sensor chips, 3-(Aminopropyl)-triethoxysilane(APTES) and bis(sulfosuccinimidyl)-suberate (BS3) were obtained from Thermo Fisher Scientific. For the hybridization steps, a high stringency hybridization buffer was made in 50 mL batches containing 15 mL of formamide (Fisher), 1 mL 10% sodium dodecyl sulfate (Fisher), 10 mL 20X saline-sodium phosphate buffer (Invitrogen), 6 mL 0.25 M ethylenediaminetetraacetic acid (Invitrogen) and 2.5 mL 50X Denhardt's solution (Invitrogen).

Instrumentation

Microring sensor arrays and measurement equipment were purchased from Genalyte, Inc. (San Diego, CA). Sensor arrays were fabricated using standard photolithography and etching techniques. The final sensor arrays are coated with a fluoropolymer coating, which is only removed from active microring sensor elements, and are diced so as to contain 132 individually-addressable microring resonator sensors. For hybridization experiments, sensor arrays are loaded into a Mylar gasket and Teflon lid are used to direct solutions into two defined flow chambers aligned with the sensor elements. Integrated pumps under software control automated all liquid handling steps.

Resonant wavelengths for each microring were determined by coupling a tunable laser source into an adjacent linear waveguide via on-chip grating couplers. The laser output was then swept through an appropriate spectral window and the light intensity at the end of the linear waveguide was used to determine the resonance wavelength. This process was serially repeated for each ring in the array, and the resultant shifts in resonance were recorded as a function of time.

The resonances supported by the microring resonator sensors meet the following mathematical condition:

$$m\lambda = 2\pi r n_{\text{eff}}$$

where m equals a nonzero integer, λ is the wavelength of propagating light supported by the microring resonator sensor, r is the microring radius, and n_{eff} is the effective refractive index of the local microring environment. Light is confined in the waveguide by total internal reflectance resulting in an evanescent field extending only a short distance from the surface

of the microring sensor. Biomolecular binding events within the region lead to a change in the resonant wavelength of the cavity, which is monitored by the optical scanning instrumentation. The binding of biomolecules results in a resonance shift to longer wavelengths: a positive shift that is listed in units of picometers (pm).

Surface functionalization

Surface functionalization was performed using one of two protocols: manually spotting by hand or automated microspotting.

Manual spotting was used in validation experiments and obtaining calibration curves. Prior to chip functionalization, chips were cleaned with a Piranha solution (70% sulphuric acid/30% hydrogen peroxide) for 30 seconds at 60°C. CAUTION: Piranha solution reacts vigorously with organics and should be handled with care. Then, the chips were rinsed with water and dried with nitrogen. Once dried, chips were immersed in acetone for 2 minutes, followed by the surface silanization with a 5% APTES solution (diluted in acetone) for 4 minutes. After silanization, the chips were immersed in acetone and isopropanol for 2 minutes each. All steps were completed with continued shaking. Chips were rinsed with water and nitrogen dried to complete the silanization process. Next, 20 µL of a freshly prepared BS3 solution (2.85 mg/mL in 2 mM acetic acid) was placed on the microring array for 3 minutes. BS3 served as the linker between the amine groups of the silanized surface and the amino-functionalized nucleic acid capture probes. After BS3 incubation, the chips were dried with nitrogen, and the final step consisted of spotting approximately 0.26 µM of 200 µM 5' amino functionalized DNA captures probes onto discrete microring sensors. The chips were then left to incubate for at least 4 hours in a humidity chamber.

Automated microspotting was used to create sensor arrays for the cross reactivity studies and clinical sample profiling. This procedure was similar to manual spotting conditions with the only differences being the use of a 1% APTES solution, a lower concentration of BS3 (1 mg/mL), and a lower concentration of the DNA capture probes (100 µM).

Reverse transcription-Asymmetric PCR amplification

Reverse transcription reactions were conducted using a TaqMan microRNA Reverse Transcription Kit. Each 15 µL reaction volume contained 4.16 µL of nuclease free water, 1.5 µL of 10X RT buffer, 1 µL of Multiscribe™ RT enzyme (50 U/µL), 0.19 µL of RNase inhibitor (20 U/µL), 0.15 µL dNTP mix (100 mM), 5 µL of RNA sample (10 ng of RNA in the case of patient samples) and 3 µL of the reverse transcription primer. The concentration of the stem loop primer was 20 µM for all experiments, except for the data presented in Figure S1 where 200 µM was used. The thermal profile was completed following the manufactures protocol: 16° C (30 min), 42° C (30 min), and 85°C (5 min).

Asymmetric PCR was performed using the Platinum Multiplex PCR Master Mix. Each 50 µL reaction volume was composed of 14 µL nuclease free water, 25 µL of Platinum® Multiplex PCR Master Mix, 5 µL of each primer and 1 µL of the reversed transcription product. The concentration of the forward primer (the limiting primer) was 2 µM while the concentration of the reverse primer was 200 µM. The reactions were incubated at 95 °C for 2 min, followed by cycles of 95 °C for 30 s, 56°C for 1 min 30 s and 72 °C for 1 min.

Sample introduction and fluidic handling

For hybridization experiments, the 50 μ L PCR reaction volume was diluted with 350 μ L of hybridization buffer and then subjected to the fluidic handling recipe outlined in Table S2.

Data analysis

Data was analyzed with Origin Pro 9.0 and completed in three steps: (1) calculation of the hybridization response; (2) determination of C(t) values; and (3) compilation of heat maps.

To calculate the hybridization response, sensor traces were first corrected for temperature and instrumental drift by using a series of reference sensors that were not in contact with the sample solution. Resonance shifts from aPCR product hybridization were calculated by subtracting the baseline buffer signal (resonance shift) at 5 minutes from the post-hybridization response (22 minutes). The shift from off-target control sensors was then subtracted from the response from miRNA-specifically-functionalized sensors.

Threshold cycle [C(t)] values were determined by plotting the net resonance wavelength shifts versus the PCR cycle number for every target (Figure 2). C(t)s were calculated in one of two ways: by either determining the point at which the second derivative of the logistic fit equaled zero (second derivative method), or the point at which 40% of the maximal signal was as the cycle number that achieved 40% of the maximum signal (linear thresholding). C(t) values were found to be linear with input miRNA concentration for all targets (Figure S1).

To compare relative expression levels of multiple miRNAs from primary tumor samples, a heat map produced by subtracting C(t) values for each miRNA measured from the primary samples from a commercial “healthy” brain total RNA sample (Thermo Fisher). The resulting values are plotted in Table S4 using a log 2 scale. Positive values represent higher expression in tumor tissue and negative values represent lower expression in tumor tissue, relative to healthy.

Cross-reactivity experiments

To probe the specificity of the capture probes, reverse transcription followed by 20 cycles of asymmetric PCR was carried out using 200 nM dilutions of all eight target miRNAs. The resulting products (50 μ L) were diluted in 350 μ L of hybridization buffer and flowed across the surface following the recipe described in Table S2.

Results and discussion

The overall workflow for this analytical scheme is shown in Figure 1. After isolation of total RNA, uniquely-designed, sequence-specific stem loop primers were used to reverse transcribe (RT) miRNAs into cDNAs.²⁹ RT products were then amplified via asymmetric PCR to generate single-stranded targets that were flowed across microring resonator arrays for label-free, hybridization-based detection. PCR primers were designed such that the limiting primer targeted the microRNA-specific region of the RT product. The primer used in excess (100x) targeted a universal region common to each target-specific stem-loop probe. Importantly, the same excess primer could be used for the entire panel of miRNA

targets. As illustrated in Figure 1, dsDNA is produced until the limiting PCR primer is exhausted, after which point additional thermal cycles create ssDNAs by extending only from the remaining excess primer. After aPCR, the product was diluted into a high stringency hybridization buffer and flowed across a sensor array for label-free, hybridization-based detection. The transition from duplex to single-strand production is dependent upon target concentration and thus directly proportional to the initial concentration of the miRNA target in the sample. The concentration of single stranded DNA product after a defined number of cycles therefore also is directly proportional to the amount of miRNA in the initial solution. In this way, the combination of stem loop primers, aPCR, and silicon photonic microring resonators can be used to performed multiplexed miRNA expression profiling.

To validate this approach, primer sets, including target-specific stem-loop sequences, were designed for eight miRNAs (sequences provided in Supplementary Information). Using synthetic miRNAs RT was first performed, followed by aPCR for defined numbers of thermal cycles. After aPCR, the resulting amplicon-containing solution was flowed across microring sensors presenting a capture sequence. Figure 2A shows the resonance shifts for the detection of a representative target (let-7f) across a range of input concentrations as a function of cycle number. Each of the concentration-dependent resonance shift data sets was fit with a logistic function for clarity. Higher concentrations of let-7f give observable resonance shifts at as few as 15 cycles with lower concentrations requiring larger numbers of cycles—i.e. logistic functions for lower concentrations were shifted to higher cycle numbers. In this way, the onset of ssDNA production and microring hybridization response was dependent upon input miRNA concentrations.

To enable quantitation of miRNA concentrations, we explored two different approaches to assign a numerical threshold cycle value associate with the hybridization response. This is analogous to $C(t)$ values from conventional RT-qPCR analysis,³⁰ and we have adopted the same naming convention. Our first approach involved assigning $C(t)$ as the value at which the second derivative of the logistic function equaled zero. A second approach involved determining the $C(t)$ value at which 40% of the maximal shift was observed via interpolation of the linear response region. The resulting calibration curves relating concentration to $C(t)$ values determined via both the 2nd derivative and linear thresholding methods for a representative target miRNA, let 7-f, are shown in Figure 2B and are in good agreement. Notably, both show dynamic ranges in excess of six orders of magnitude in target concentration. For the second derivative method, error bars come from the logistic fit parameter estimate variance. For the linear thresholding approach, error bars were generated by propagating the error from measurements from the array of technical replicate sensors. Similar calibration curves were obtained for the other miRNA targets in the multiplexed panel (see Supporting Information).

Target specificity is obviously of high importance in miRNA analysis. In order to test specificity of this combined aPCR and microring resonator method, RT-aPCR amplification was performed separately for each of the eight targets with each amplicon-containing solution flowed over an array of microring sensors functionalized with capture probes against each specific target. As shown in Figure 3, hybridization responses are only observed

for sensors functionalized with the appropriate sequence-specific capture probes, demonstrating a high degree of specificity.

Following the validation and calibration of this approach, we applied it to the simultaneous profiling of eight miRNA targets from total RNA extracted from twenty primary surgical brain tumor specimens, as well as a commercially “healthy” brain total RNA sample. RT followed by aPCR at defined cycle numbers was performed before hybridization analysis on the multiplexed microring sensor array. Data from the healthy total RNA sample and a representative glioma specimen (Subject A) are shown in Figure 4A–B, respectively, as a function of cycle number. C(t) determination was achieved via the linear thresholding approach.

For visual comparison of relative fold changes²⁹ between the mRNA expression levels in the twenty brain tumor samples relative to the healthy control, the sensor responses were compiled into a heat map, shown in Figure 5. Differential miRNA expression is observed across the data set with some miRNAs showing higher expression (red) and other lower abundance (blue). Tumor samples are clearly heterogeneous and so it is important not to draw too substantial conclusions from this initial data. However, there are several observations that are at least partially consistent with literature precedent. For example, miRs-10b, 155, and 222 have been reported to be upregulated in some gliomas.^{30–32} By contrast, miRs-34a and 29a have been reported as downregulated, again, in some gliomas.^{33–34} Larger scale miRNA expression profiling efforts might help identify important biomarkers with diagnostic utility for some tumor specimens/subtypes and the described microring resonator approach, coupled with aPCR, is a potentially valuable method for measuring targeted miRNA panels from larger research subject cohorts given the inherent scalability of the technology.

Conclusions

In summary, the integration of asymmetric PCR and silicon photonic microring resonator arrays resulted in the successful profiling of miRNAs from surgical glioma specimens with expression levels consistent with literature precedent. This approach leverages the aPCR to selectively amplify miRNA targets using a new stem loop primer approach while enhancing multiplexing capacity over traditional qPCR methods through the use of label-free hybridization detection on using microring resonator arrays. We describe two methods for analyzing the hybridization responses as a function of cycle number and demonstrate that the conversion from duplex to single-strand amplification correlates with input target concentration. Importantly, this integrated approach is promising for targeted panel-based miRNA diagnostic approaches that continue to gain traction for applications in large cohort biomarker discovery. Future work that integrates together thermocycling with real-time, on-line hybridization measurements using sensor arrays could substantially lower reagent consumption and input requirements for multiplexed miRNA expression profiling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Liotta L, Petricoin E. *Nat Rev Genet.* 2000; 1:48–56. [PubMed: 11262874]
2. Hanash SM, Baik CS, Kallioniemi O. *Nat Rev Clin Oncol.* 2011; 8:142–150. [PubMed: 21364687]
3. Pritchard CC, Cheng HH, Tewari M. *Nat Rev Genet.* 2012; 13:358–369. [PubMed: 22510765]
4. Calin GA, Croce CM. *Cancer Res.* 2006; 66:7390. [PubMed: 16885332]
5. Madrigal-Matute J, Rotllan N, Aranda JF, Fernández-Hernando C. *Curr Atheroscler Rep.* 2013; 15:322. [PubMed: 23512606]
6. Vickers Kasey C, Rye K-A, Tabet F. *Clin Sci.* 2013; 126:183.
7. Zhu S, Pan W, Qian Y. *J of Mol Med.* 2013; 91:1039–1050. [PubMed: 23636510]
8. Abe M, Bonini NM. *Trends Cell Biol.* 2013; 23:30–36. [PubMed: 23026030]
9. Fan Y, Siklenka K, Arora SK, Ribeiro P, Kimmins S, Xia J. *Nucleic Acids Res.* 2016; 44:W135–W141. [PubMed: 27105848]
10. Graybill RM, Bailey RC. *Anal Chem.* 2016; 88:431–450. [PubMed: 26654257]
11. Carrascosa LG, Huertas CS, Lechuga LM. *TrAC Trend Ana Chem.* 2016; 80:177–189.
12. Henriksen M, Johnsen KB, Andersen HH, Pilgaard L, Duroux M. *Mol Neurobiol.* 2014; 50:896–913. [PubMed: 24619503]
13. Iqbal M, Gleeson MA, Spaugh B, Tybor F, Gunn WG, Hochberg M, Baehr-Jones T, Bailey RC, Gunn LC. *IEEE J Sel Top Quantum Electron.* 2010; 16:654–661.
14. Washburn AL, Gunn LC, Bailey RC. *Anal Chem.* 2009; 81:9499–9506. [PubMed: 19848413]
15. Wade JH, Alsop AT, Vertin NR, Yang H, Johnson MD, Bailey RC. *ACS Central Science.* 2015; 1:374–382. [PubMed: 26539563]
16. Sloan CDK, Marty MT, Sligar SG, Bailey RC. *Anal Chem.* 2013; 85:2970–2976. [PubMed: 23425255]
17. Kindt JT, Bailey RC. *Anal Chem.* 2012; 84:8067–8074. [PubMed: 22913333]
18. Qavi AJ, Myszyk TM, Bailey RC. *Anal Chem.* 2011; 83:6827–6833. [PubMed: 21834517]
19. McClellan MS, Domier LL, Bailey RC. *Biosens and Bioelectron.* 2012; 31:388–392.
20. Qavi AJ, Bailey RC. *Angew Chem Int Ed.* 2010; 49:4608–4611.
21. Qavi AJ, Kindt JT, Gleeson MA, Bailey RC. *Anal Chem.* 2011; 83:5949–5956. [PubMed: 21711056]
22. Graybill RM, Para CS, Bailey RC. *Anal Chem.* 2016; 88:10347–10351. [PubMed: 27726377]
23. Pollet J, Janssen KPF, Knez K, Lammertyn J. *Small.* 2011; 7:1003–1006. [PubMed: 21394905]
24. Gyllenstein UB, Erlich HA. *Proc Natl Acad Sci USA.* 1988; 85:7652–7656. [PubMed: 3174659]
25. Sanchez JA, Pierce KE, Rice JE, Wangh LJ. *Proc Natl Acad Sci USA.* 2004; 101:1933–1938. [PubMed: 14769930]
26. Kai E, Sawata S, Ikebukuro K, Iida T, Honda T, Karube I. *Anal Chem.* 1999; 71:796–800. [PubMed: 10051847]
27. Zhu LX, Zhang ZW, Liang D, Jiang D, Wang C, Du N, Zhang Q, Mitchelson K, Cheng J. *Antimicrob Agents and Ch.* 2007; 51:3707–3713.
28. Chung HJ, Castro CM, Im H, Lee H, Weissleder R. *Nat Nanotechnol.* 2013; 8:369–375. [PubMed: 23644570]
29. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ, Livak KJ, Guegler KJ. *Nucleic Acids Res.* 2005; 33:e179. [PubMed: 16314309]

30. Schmittgen TD, Livak KJ. Nat Protocols. 2008; 3:1101–1108. [PubMed: 18546601]
31. Lin J, Teo S, Lam DH, Jeyaseelan K, Wang S. Cell Death Dis. 2012; 3:e398. [PubMed: 23034333]
32. Zhou J, Wang W, Gao Z, Peng X, Chen X, Chen W, Xu W, Xu H, Lin MC, Jiang S. PLOS ONE. 2013; 8:e83055. [PubMed: 24376632]
33. Quintavalle C, Garofalo M, Zanca C, Romano G, Iaboni M, del Basso De Caro M, Martinez-Montero JC, Incoronato M, Nuovo G, Croce CM, Condorelli G. Oncogene. 2012; 31:858–868. [PubMed: 21743492]
34. Yin D, Ogawa S, Kawamata N, Leiter A, Ham M, Li D, Doan NB, Said JW, Black KL, Phillip Koeffler H. Oncogene. 2013; 32:1155–1163. [PubMed: 22580610]

Figure 1.

(A) Overview of the combined aPCR-microring assay. Isolated RNA is reverse transcribed and asymmetrically amplified to single strand products that are detected via hybridization onto the sensor array. (B) Schematic illustration of reverse transcription using stem loop probes and aPCR amplification. (C) Plot showing DNA amplification as a function of increasing cycle number. Double-stranded product is made until the limiting primer is consumed, after which further cycling yields single-stranded products. (D) Resonance wavelength shifts are only detected after the transition to single-stranded production, which allows amplicons to hybridize to capture sequences arrayed onto unique microring sensor elements.

Figure 2.

(A) Resonance wavelength shifts for various concentrations of let-7f as a function of cycle number. Error bars are the standard deviation from $n = 8$ microrings. Each cycle-dependent trace is fit with a logistic function. (B) Concentration-dependent calibration curves for let-7f determined using both the 2nd derivative and linear thresholding methods.

Figure 3. Cross reactivity experiments showing target-specific responses as each single miRNA aPCR product is flowed across a sensor array.

Figure 4.

Resonance wavelength simultaneously measured by an array of microrings presenting capture probes for eight miRNAs after performing aPCR and hybridization analysis from a 10 ng input sample of (A) healthy brain total RNA, and (B) a representative primary glioma specimen (Subject A). (A–B) Results obtained when using a 10 ng input of a healthy control and glioma grade IV total RNA sample, respectively, and subjecting it to varying cycles of the aPCR-microring assay.

Figure 5.

Heat map showing expression profiles from patient samples, relative to healthy control brain total RNA. Higher expression is indicated in red and lower in blue with numerical values plotted on a log2 scale.