Label-free and amplification-free miR-124 detection in human cells

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Abstract. MicroRNAs (miRNAs) are becoming a very important group of molecules especially since their connection to numerous diseases has been revealed. The potential in gene therapy as well as in diagnostics is being widely investigated leading to the demand of sensitive, selective and simple methods of isolation and detection. The combined advantages of magnetic particle-based separation with sensitive electrochemical detection may offer a very valuable tool for these purposes. In this study, the miR-124 was targeted as an example analyte for development and optimization of the isolation procedure coupled to the electrochemical detection. The sensitivity of the method was demonstrated by the limit of detection at the level of nanomolar concentration (4 nM). To verify the applicability of the procedure to the real samples, miR-124 was isolated from the human embryonic kidney cells naturally expressing this miRNA molecule and the results were compared to the amount of miR-124 isolated from the cells transfected by the pENTR-miR-124 plasmid leading to the overexpression of miR-124.

Introduction

MicroRNAs (miRNAs) are a novel class of evolutionary conserved single-stranded RNAs, which have an important role in the regulation of gene expression at the posttranscriptional level. These short non-coding RNAs (~23 nucleotides long) were discovered in 1993 (1) and since that time 24,521 entries from over 200 species leading to production of >30,000 mature miRNAs have been found (June 2013, miRBase.org). MiRNA biogenesis is a multilevel process involving many enzymes and proteins; however its regulation is quite different from the previously described regulators of gene expression. Within the canonical model of biogenesis, genes for miRNAs contain their own promoters and are transcribed by RNA polymerase II into primary transcripts (pri-miRNA) (2,3). Thus unique way of transcription predestines them to have unique properties. An effect of miRNAs is most often based on the binding to the untranslated region (3'UTR) of target mRNA causing degradation (or inhibition) of target mRNA. It is not surprising that they influence numerous cellular processes such as proliferation, differentiation, apoptosis, metastases, angiogenesis, and immune response (4,5), of these many are connected with diseases including tumor ones. It has been found that miRNAs may have a different expression pattern in a patient with a tumor disease in comparison to healthy subjects, whereas many miRNAs are specific for a given type of cancer (6). Recently, many studies have shown that miR-124, usually expressed in the developing nervous system, is down-regulated in several types of cancers such as breast cancer (7,8), hepatocellular carcinoma (9), lymphoblastic leukemia (10) and prostate cancer (11). MiR-124 also contributes to the differentiation of neurons (12), regulates proliferation (13) and gastrulation of stem cells (14).

The detection and quantification of miRNAs is very important for the gene expression profiling, however, there are several limitations of miRNAs detection such as their short length and tissue-specific occurrence. Basic methods used for detection are northern blotting, real-time reverse transcription polymerase chain reaction (RT-qPCR), *in situ* hybridization (ISH) and micro-RNA arrays (15-22). These methods require labelling (radioactive, fluorescent), amplification and/or enzymatic catalysis. With the exception of the RT-qPCR none of these techniques is quantitative. Besides these methods, electrochemical (EC) methods can be also used (23). From the EC methods, those detecting reduction of nucleic acids bases on mercury electrodes belong to the most sensitive ones. Palecek was the first who used modern

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oscillographic polarography for successful detection of redox DNA signals (23,24). Since then, attention is paid to various electrochemical methods including linear sweep and cyclic polarography/voltammetry (elimination polarography/voltammetry), differential pulse polarography/voltammetry, square wave polarography/voltammetry, AC polarography/voltammetry, and chronopotentiometry for analysis of DNA (23,25). Square wave voltammetry (SWV) is one of the most sensitive EC methods for determination of oligonucleotides (ODNs) (26). SWV offers background suppression combined with the effectiveness of differential pulsed voltammetry (DPV), slightly greater sensitivity compared to DPV, much faster scan rates, and applicability to a wider range of electrode materials and systems. The most reproducible behavior and the lowest detection limits are generally found at mercury surfaces (23). For the simple, low-cost and sensitive detection the EC analysis was linked with nanomaterials. Among nanoparticles (NPs) which can be used in connection with label-based EC methods belong the OsO_2 NPs, RuO_2 NPs, gold NPs or magnetic particles (MPs) (27-31). On the contrary, for the label-free miRNA detection in connection with EC methods silicon nanowires, silver or gold nanostructures and carbon nanotubes can be used (32-35).

In this study, we coupled electrochemical analysis with MPs-based extraction, which does not need any specific pretreatment, for detection of miR-124 in cell extracts.

Materials and methods

Chemicals. All chemicals of ACS purity were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. The 1X binding and washing (BW) buffer (5 mM Tris-HCl, 0.5 mM EDTA and 1.0 M NaCl, pH 7.5), solution A (0.1 M NaOH and 0.05 M NaCl) and solution B (0.1 M NaCl) were employed for MPs washing. For biotinylated anti-miR-124 immobilization, the 2X BW buffer (10 mM Tris-HCl, 1 mM EDTA and 2.0 M NaCl, pH 7.5) was utilized. The phosphate buffer I for washing MPs with immobilized oligonucleotide was composed of 0.1 M NaCl, 0.05 M Na_2HPO_4 and 0.05 M NaH_2PO_4, pH 7.8.

The composition of hybridization solution was as follows: 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, 0.6 M guanidinium thiocyanate (Amresco, Solon, OH, USA), 0.15 M Tris-HCl and 0.5 M NaCl (pH 7.5). The elution solution composition was: 0.2 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. All solutions were treated with diethylpyrocarbonate (DEPC) or prepared in DEPC treated water. Acetate buffer (0.2 M CH_3COOH and 0.2 M CH_3COONa, pH 5.0) was used for electrochemical analysis.

The miR-124-3p (5'-UAA GGC ACG CGG UGA AUG CC-3') and complementary biotinylated oligonucleotide (ODN) anti-miR-124-3p (5'-Btn-GG CAT TCA CCG CGT GCC TTA-3', both synthesized by Sigma-Aldrich, were used for magnetic separation optimization. For the binding specificity confirmation, ODNs of the following sequences were used: ODN 10 (ATGGCAGACA), ODN 21 (GCGATTGATG GTGATACGGTT), ODN 55 (GGGGACAAGTTTGTACA AAAAAGCAGGCTGTGGCTAATACGAAAAAAACAAC ATT) and miR-150 (UCU CCC AAC CCU UGU ACC AGU G). The ODNs were also synthesized by Sigma-Aldrich.

MiR-124 isolation by magnetic particles. The isolation procedure was carried out according to the scheme shown in Fig. 1. The magnetic microparticles Dynabeads M-270 Streptavidin (Life Technologies, Invitrogen, Oslo, Norway) and magnetic separation rack MagnaRack (Life Technologies, Invitrogen) were used for miRNA isolation. The miRNA experiments were performed in RNA/DNA UV-cleaner box UVT-S-AR (Biosan, Riga, Latvia) as follows. The biotinylated anti-miR-124 immobilization on MPs surface was done according to the manufacturer's recommendations. Briefly, a microcentrifuge tube with 50 μl of resuspended MPs was placed on the magnetic rack. After 1-2 min the supernatant (storage solution) was removed and the washing step followed. The tube was taken out from magnetic stand and the MPs were resuspended by pipette in 50 μl of 1X BW buffer. Then, the tube was returned to magnetic rack and the supernatant was removed. The washing process was repeated 3 times using BW buffer. Subsequently, the MPs were washed the same way twice with 50 μl of the solution A and once with the solution B (50 μl).

The washed MPs were ready for immobilization of biotinylated ODN. MPs were resuspended in 100 μl of 2X BW buffer and the amount of added biotin anti-miR-124 was optimized. The used volume (1.5, 2, 2.5, 3 and 3.5 μl) of 100 μM biotinylated anti-miR-124 was diluted in DEPC-treated water reaching the final volume of 200 μl. The mixture with MPs and biotinylated ODN was incubated for 10 min on rotator-mixer (multi RS-60, Biosan) at 60 rpm at room temperature. The biotinylated anti-miR-124 was bound during the incubation to streptavidin on MPs surface. After the incubation, the coated MPs were separated on the magnetic rack and twice washed with 50 μl of 1X BW buffer.

Further, the MPs were twice washed with 100 μl of phosphate buffer I and the hybridization step was performed according to Huska *et al* (36). Briefly, 50 μl of hybridization solution and 50 μl of sample (miR-124 diluted in water) were added to the anti-miR-124 coated MPs. The hybridization process took place on a rotator-mixer at 60 rpm for 40 min at room temperature. During this step, the miR-124 was bound to complementary anti-miR-124 on MPs surface. After the incubation, the tube was placed on magnetic rack, the hybridization solution was removed and the MPs with miR-124 were washed three times with 100 μl of phosphate buffer I (36).

Moreover, the MPs were resuspended in 50 μl of the elution solution. The miR-124 elution was done in Thermomixer 5355 Comfort/Compact (Eppendorf, Hamburg, Germany) for 5 min at 350 rpm, whereas the elution temperature was optimized. The used temperatures were within the range from 50 to 90˚C. During the elution, higher temperatures were used and this caused double-stranded RNA (dsRNA) denaturation and the miRNA was released from MPs surface. Further, the tube was placed on a magnetic stand and the solution with eluted miR-124 was pipetted to a new tube. The miR-124 amount was electrochemically determined.

Electrochemical analysis. Electrochemical measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Utrecht, The Netherlands) connected to VA-Stand 663 (Metrohm, Zofingen, Switzerland) using a standard cell with three electrodes. A hanging mercury drop

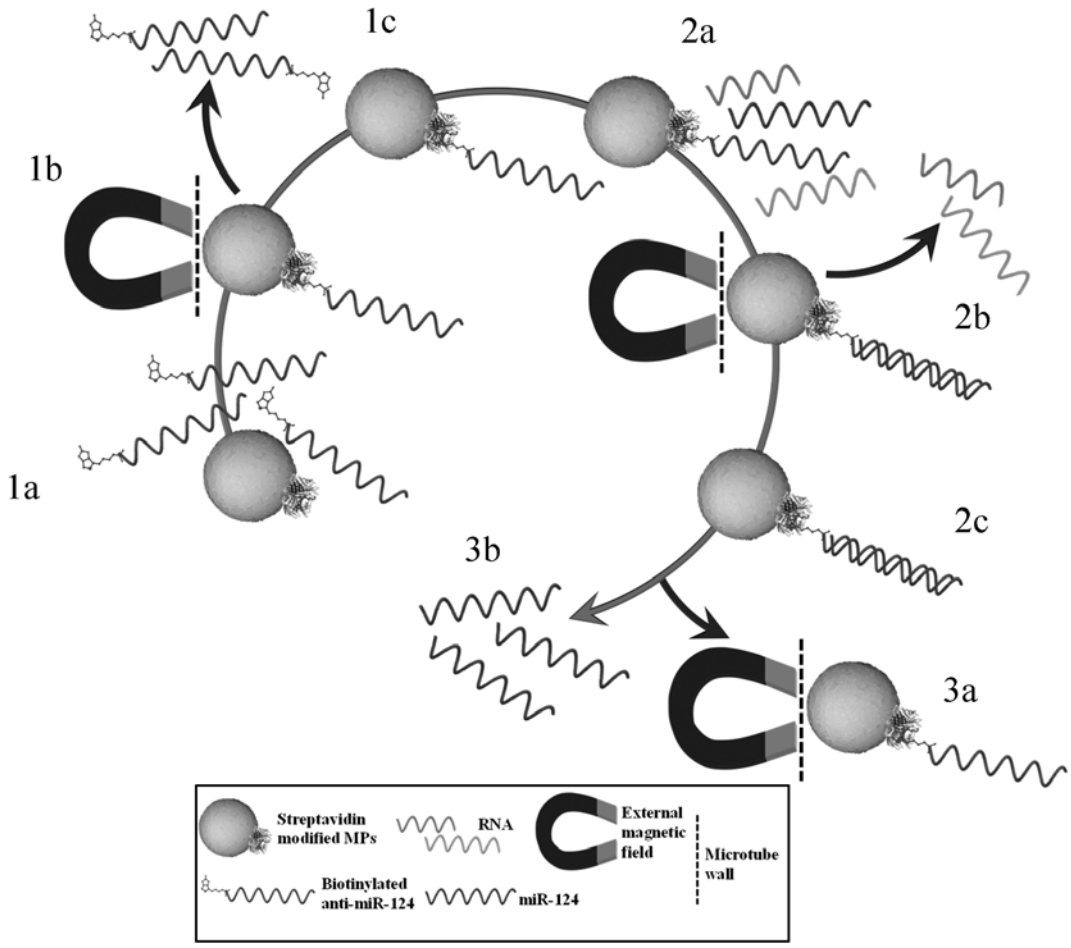

Figure 1. The magnetic separation scheme. (1) The first step is immobilization of biotinylated anti-miR-124 to streptavidin modified MPs surface. (1a) The anti-miR-124 incubation with MPs. (1b) The separation of anti-miR-124 coated MPs by the magnet and the excess unbound anti-miR-124 removal. (1c) The anti-miR-124 coating MPs. (2) The second step is miR-124 hybridization to anti-miR-124 modified MPs. (2a) The sample incubation with MPs. (2b) The separation of miR-124 coated MPs by the magnet and the non-complementary RNA removal. (2c) The miR-124 coupling with anti-miR-124 modified MPs. (3) The third step is miR-124 elution from MPs surface. (3a) The MPs separation by the magnet. (3b) The isolated miR-124.

electrode (HMDE) with a drop area of 0.4 mm^2 was employed as the working electrode. An Ag/AgCl/3M KCl electrode served as the reference electrode. Pt electrode was used as the auxiliary electrode.

Adsorptive transfer technique was used for the electrochemical determination of RNA. The adsorptive transfer technique is based on the sample accumulation (120 s) onto the working electrode surface, washing of the electrode and square wave voltammetric (SWV) measurement. All experiments were carried out at room temperature (21˚C). The SWV conditions were performed according to Hynek *et al* (37). SWV measurements were carried out in the presence of 0.2 M acetate buffer pH 5.0. SWV parameters were as follows: start potential 0 V, end potential -1.8 V, potential step 5 mV, frequency 280 Hz, and amplitude 25.05 mV. For smoothing and baseline correction, the software GPES 4.9 supplied by EcoChemie was employed.

Plasmid construction. pENTR-miR-124 plasmid was constructed from pENTR/U6 (Life Technologies, Rockville, MD, USA) where mouse U6 promoter was replaced with human U6 promoter followed by two *Bsa*I restriction sites. These sites were used for cloning of miRNA precursor. The insert was created by annealing two synthetic oligonucleotides as mi124F-Bsa: CCTGGTCTCACACCGGGCATTCACCGCG TAACTTATTCAAGAGATAAGGCACGCGGTGAATGCC TTTTTGAGACAGG, and mi124R-Bsa: CCTGGTCTCAAA AAGGCATTCACCGCGTGCCTTATCTCTTGAATAAGTT ACGCGGTGAATGCCCGGTGTGAGACCAGG. Annealed oligonucleotides were digested with *Bsa*I and cloned into vector cleaved with the same enzyme.

Preparation of miR-124 enriched RNA. HEK293 cells were cultivated in DMEM media (PAA) containing 10% FBS (fetal bovine serum). Cells were transfected with pENTR-miR-124 plasmid with Polyethyleneimine MAX (Polysciences, Eppelheim, Germany). Forty-eight hours after transfection, the cells were washed with PBS and RNA was extracted with TRIzol reagent (Life Technologies) according to the manufacturer's protocol. The isolated RNA was dissolved in DEPC-treated water.

RNA isolation. RNA isolation from cells was done using TRIzol method. Briefly, 200 μl of TriPure reagent (Roche, Mannheim, Germany) was applied on the cells ($2x10^6$) and these were incubated for 5 min at room temperature. Then,

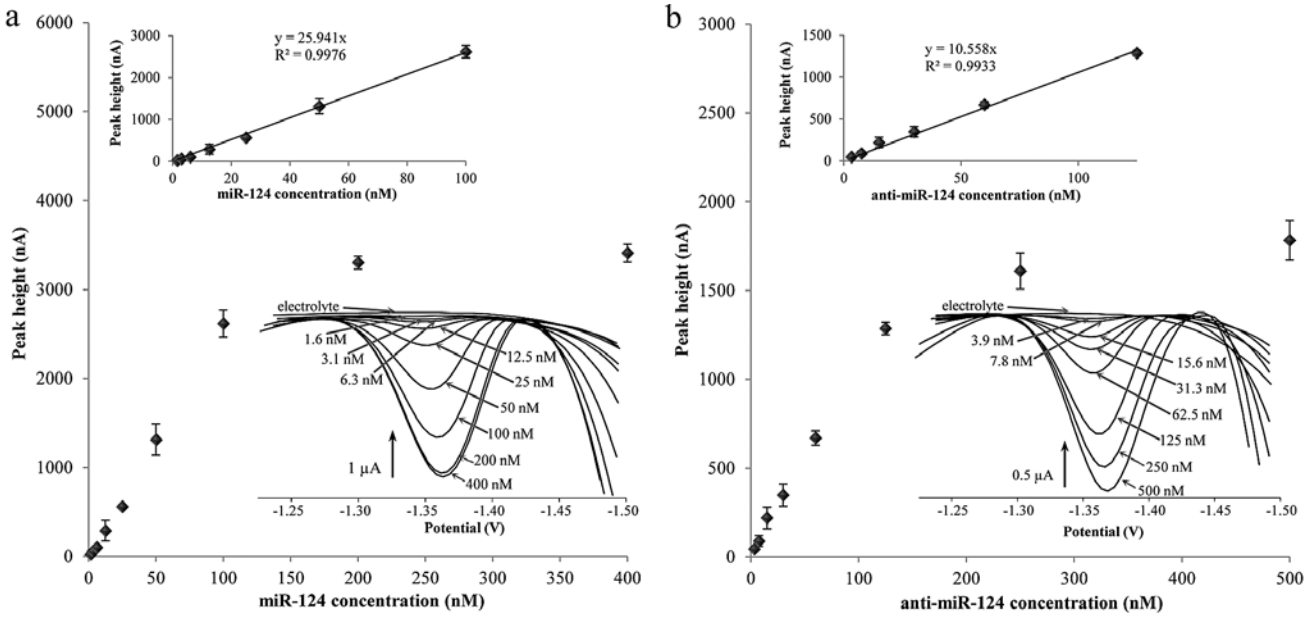

Figure 2. SWV peak height dependence on concentration of (a) miR-124 (upper inset, the linear range of calibration curve; lower inset, the voltammograms of used miR-124 concentrations) and (b) anti-miR-124 (upper inset, the linear range of calibration curve; lower inset, the voltammograms of used anti-miR-124 concentrations). SWV parameters were as follows: time of accumulation 120 sec start potential 0 V, end potential -1.8 V, potential step 5 mV, frequency 280 Hz, amplitude 25.05 mV.

40 μl of chloroform was added followed by centrifugation at 12,000 x g for 15 min at 4˚C. After the centrifugation, RNA was in the upper aqueous phase, which was removed and transferred to an RNase-free tube. Subsequently, 100 μl isopropanol was added to precipitate RNA from the solution. The precipitated samples were incubated at 25˚C for 10 min followed by centrifugation of the samples at 12,000 x g for 10 min at 4˚C, after which the supernatant was discarded and the pellet washed with 200 μl of 75% ethanol (v/v). Moreover, the samples were mixed using vortex and centrifuged at 7500 x g for 5 min at 4˚C. After centrifugation, the supernatant was removed, pellets dried and dissolved in 50 μl of RNase-free water in a thermostat at 58˚C for 18 min.

Real-time reverse transcription quantitative polymerase chain reaction. The isolated miRNA was firstly converted into cDNA by reverse transcription, for which the TaqMan MicroRNA Reverse Tranciption kit (Applied Biosystems, Foster City, CA, USA) with a miRNA-specific primer TaqMan MicroRNA Assays for hsa-miR-124-3p (Applied Biosystems) was used. Briefly, 10 ng of total RNA was used for 15 μl reaction with 3 μl of specific primer (71.4 nM) and 7 μl of mastermix.

Real-time reverse transcription quantitative polymerase chain reaction (real-time qRT-PCR) was performed in triplicates using the TaqMan gene expression assay system with the 7500 real-time PCR system (Applied Biosystems), and the amplified cDNA was analyzed by the comparative Ct method using sample without plasmid as an endogenous control and for expression quantification. The specific fluorescent primer probe for quantification of hsa-miRNA-124-3p was selected from TaqMan miRNA expression assays (Applied Biosystems). qPCR was performed under the following amplification conditions: total volume of 20 μl, initial denaturation 95˚C/10 min, then 45 cycles 95˚C/15 sec, 60˚C/1 min. For the evaluation, we selected value 1 for expression cycle (CT) of the basic sample (without plasmid), and from it we derived values for samples with inserted plasmids. The evaluation was performed using MS Excel.

Structural prediction. The structure of miR-124 as well as anti-miR-124 was predicted using a freeware software Oligo analyzer (https://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/) enabling us calculation of the free Gibbs energy for given sequences. Input parameters were as follows: oligo concentration 0.006-0.400 μM (concentrations of miR-124 applied for recovery determination), and Na^+ concentration 800 mM (total concentration of sodium in the hybridization solution, see Chemicals in Materials and methods).

Mathematical treatment of data and estimation of detection limits. Mathematical analysis of the experimental data and the graphical interpretation were carried out by the Microsoft Office tools (MS Excel®, MS Word®, and MS PowerPoint®). All results were expressed as a mean ± standard deviation (SD) unless noted otherwise. The detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner (38), whereas N was expressed as a standard deviation of noise determined in the signal domain unless stated otherwise. Calculation of the recovery was carried out as indicated by Causon (39).

Results and Discussion

It has to be noted that the electrochemical detection by itself, even though highly sensitive, lacks the sequence specificity, which would be required for determination of miRNAs belonging to the particular disease, however, its combination with the specific miRNA isolation based on highly specific

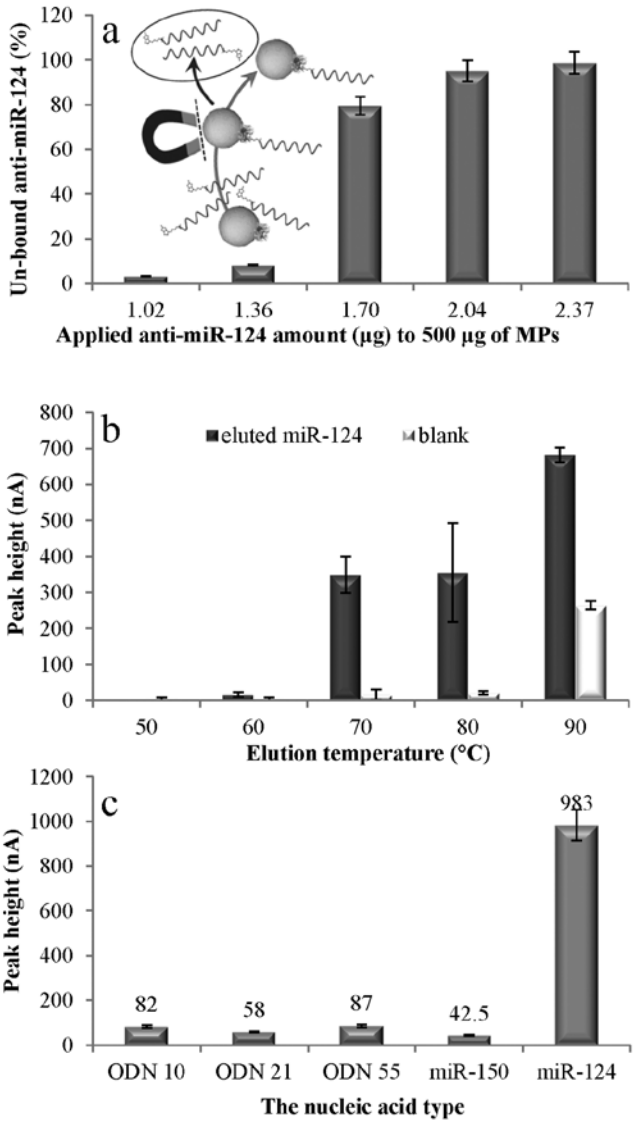

Figure 3. (a) The MP binding capacity (anti-miR-124) optimization. The dependence of biotinylated anti-miR-124, which was not bound to MPs, on the applied anti-miR-124 concentration. Inset, after anti-miR-124 immobilization MPs were separated and the unbound anti-miR-124 (in the red circle) was determined. (b) The peak height dependence of eluted miR-124 amount and/or the eluate without miR-124 on elution temperature. (c) The binding specificity verification. The peak height dependence of the eluted target oligonucleotides on target nucleotide sequence. The non-complenmentary ODNs were ODN 10, ODN 21, ODN 55, miR-150 and the complementary was miR-124.

base paring creates a very powerful technique. Moreover, addition of the advantages of magnetic particles, extremely suitable for simple sample clean up, is increasing the benefits even more.

Calibration curves of microRNA and antisense microRNA. To evaluate the SWV sensitivity, the calibration curves for miR-124 and biotinylated anti-miR-124 were determined. The measured data are shown in Fig. 2. The dependence of peak height on miR-124 concentration measured by SWV is shown in Fig. 2a. The lower inset shows the linear range of this technique. Higher miR-124 concentration than 100 nM caused HMDE surface saturation by nucleic acid and therefore 100 nM is the highest concentration showing linearity. The limit of detection was determined as 1 nM and limit of quantification was established as 4 nM. The typical miR-124 voltammograms are shown in the upper inset in Fig. 2a. In addition, the calibration curve of biotinylated antisense oligonucleotide (ODN) is shown in Fig. 2b. The SWV voltammograms of anti-miR-124 are in the upper inset. The linear range of anti-miR-124 measured by SWV is shown in the lower inset. In both cases of the calibration curve the intercept was set to 0 due to its statistical insignificance. The HMDE surface saturation by ODN occurs at higher concentration than 125 nM. The limit of detection is 1 nM and the limit of quantification is 4 nM, which is the same as that for miR-124.

The optimization of magnetic separation. For optimal MPs utilization, the binding capacity of the MP surface was determined. The anti-miR-124 was added to the MPs and after the immobilization step, and MP separation (inset in Fig. 3a), the amount of unbound antisense ODN in retantate (a solution remaining after MPs immobilization on the vial wall by magnetic field) was determined. The dependence of anti-miR-124 amount remained in the retentate on applied concentration to MPs is shown in Fig. 3a. When 1.5 μl of 100 μM probe (1.02 μg) was added the unbound probe, the determined amount was only 3.3%. With the increasing probe amount applied to the particles the unbound anti-miR-124 amount increased considerably. When 3.5 μl of 100 μM probe (2.37 μg) was added, the amount of unbound anti-miR-124 was 98.7%. Thus, the addition of 1.02 μg of anti-miR-124 to the 500 μg of MPs was used in the following experiments.

The next step of optimization was the elution temperature (Fig. 3b). During the elution, dsRNA denaturation occurs and the miR-124 is released into the elution solution. The goal was to find such a temperature, at which maximum miR-124 amount would be released and simultaneously prevent damage to streptavidin-biotin binding. At 50 and 60˚C very low miR-124 elution (the peak height 0-16 nA) occurred. An increase of the temperature to 70 and 80˚C substantially higher miR-124 yield (the peak height 349 and 356 nA, respectively) was reached. However, in the case of 80˚C, the repeatability was significantly worse (Fig. 3b). The temperature increase up to 90˚C caused another miR-124 yield growth. However, at this elution temperature significant increase of SWV anti-miR-124 signal was observed. During this experiment the control solutions were prepared containing no miR-124. It was observed that up to the elution temperature of 80˚C the anti-miR-124 signals were insignificant. Nevertheless, at 90˚C the signal grew markedly (Fig. 3b). This is probably caused by the degradation of the particle surface leading to the release of the immobilized anti-miR-124 ODN. Therefore, the elution temperature of 70˚C was used in the following experiments.

The selectivity of optimized method was verified using oligonucleotides of different lengths as well as miR-150, which has no complementarity to anti-miR-124 oligonucleotide. The oligonucleotide lengths were 10 nt (ODN 10), 21 nt (ODN 21) and 55 nt (ODN 55). As it shown in Fig. 3c, the SWV signal obtained, when the complementary sequence (miR-124) was isolated, was on average nearly 15 times higher than for the non-complementary sequence signals, which were at the same level as the blank solutions. This significantly higher extrac-

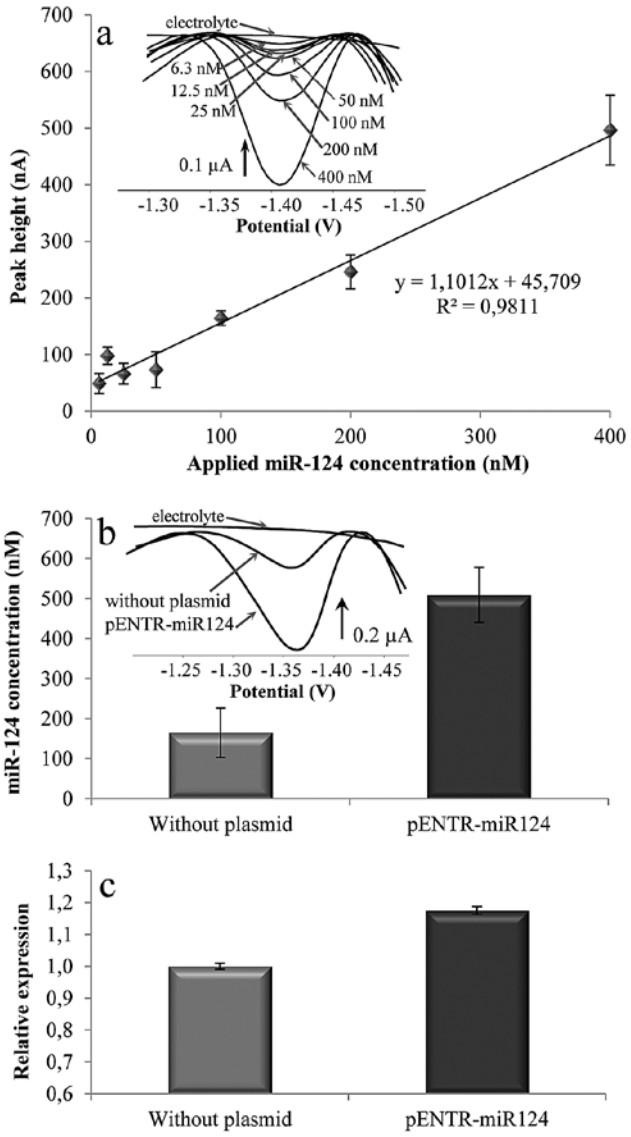

Figure 4. (a) The dependence of the peak height on applied miR-124 concentration using the optimized MPs-based isolation. Inset, the isolated miR-124 voltammograms (SWV conditions as in Fig. 1). (b) Concentration of miR-124 in non-transfected and transfected cells determined by optimized MP-based isolation procedure with SWV detection. Inset, the isolated miR-124 voltammograms (SWV conditions as in Fig. 1). (c) The miR-124 relative expression in non-transfected cells and pENTR-miR124 transfected cells (RT-qPCR).

tion efficiency confirms the selectivity towards the targeted miRNA.

To determine the sensitivity of the optimized separation method a calibration curve for the analytical process including isolation procedure and SWV determination was performed (Fig. 4a). The SWV response is linear with determination coefficient $R^2 = 0.9811$ within this range. The intercept was not set to 0 due to its statistical significance. This is caused by the fact that the calibration curve includes the whole MP-based isolation process (probe immobilization, miR-124 hybridization, elution, and EC detection) and not only the detection part as shown in Fig. 2. The limit of detection was determined as 4 nM and limit of quantification was established as 14 nM. The isolated miR-124 voltammograms are shown in the inset in Fig. 4a. To obtain the concentration of miR-124 extracted from these samples as well as the recovery calculation the regression equation shown in Fig. 2a was used. It was found out that the recovery is highly dependent on the miRNA concentration in the sample. The extraction efficiency of approximately 28.8±2.5% was reached for miRNA concentrations 0-20 nM. For the higher miRNA concentrations a higher amount of magnetic particles has to be used. The dramatic decrease in the recovery (5.4±0.8%) in case of a higher concentration of miRNA (50-400 nM) may be caused by the formation of secondary structures of both miR-124 as well as anti-miR-124. According to the prediction calculations the tendency to hairpin formation is relatively high. According to the sequence of miR-124 five potential hairpin structures may be formed with Gibbs free energies within the range from -7.23 to -3.75 kcal/mol. Anti-miR-124 has potential to form two hairpins with calculated Gibbs free energies -4.2 and -3.78 kcal/mol. Larger negative value for Gibbs free energy indicates stable hairpins preventing the correct hybridization between miR-124 and anti-miR-124.

The cell samples analysis. After the optimization and validation of the magnetic separation procedure using synthetic miRNA, the method was applied on RNA extracted from cell samples. HEK293 cells and the same cells transfected with pENTR-miR-124 ectopically expressing miR-124 were used as testing cell sample. Plasmid pENTR-miR-124 expresses miRNA in a form of short hairpin cloned under the control of U6 promoter. The hairpin is post-transcriptionally cleaved giving rise to mature miR-124. The sample of total RNA extracted from cell lines was hybridized with anti-miR-124 modified-MPs following previously described protocol. After miR-124 isolation the electrochemical detection was performed, and the results are shown in Fig. 4b (the voltammograms of isolated miR-124 are shown in inset). The SWV signal determined in non-transfected HEK293 cells correspond to 164.8 nM of miR-124. In the pENTR-miR-124 transfected cells concentration of 509.3 nM miR-124 was determined. The miR-124 concentration was 3X higher in transfected cells. The ectopic expression of miRNA from the plasmid was confirmed by northern blot hybridization (data not shown).

For the confirmation of results obtained by magnetic separation method in connection with electrochemical detection real-time RT-qPCR analysis was performed. The miR-124 relative expression was analyzed using the total RNA extracted from HEK cells (non-transfected and transfected). The results are shown in Fig. 4c. The the same trend is observed since the cells with the inserted plasmid for miR-124 increased production of these miRNAs to a greater extent than the cells without the plasmid. Even though the increase in the amount of miR-124 expressed by the HEK cells is not as high as it would be expected from the transfected cells, probably due to the un-optimized cloning/transfection procedure, this was not the main aim of the presented study. We were aiming at development of a combination of isolation and detection technique enabling simple and easy miRNA analysis.

In conclusion, exploitation of the connection between miRNA expression and disease development and progression may potentially improve our diagnostic power and moreover the application for therapeutic purposes to improve the success of treatment. Therefore, the attention attracted

by miRNAs is exhibiting continuously growing trend and it can be anticipated that the search for methods enabling easy and sensitive detection will be required. Due to the flexibility of surface modification of magnetic particles and sensitivity of electrochemical detection, the combination of these two steps is extremely beneficial. This study demonstrated the applicability of the relatively simple procedure for isolation of these very important biomolecules and their detection without requirement of complex and costly PCR-based techniques.

Acknowledgements

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