

EXPERIMENTAL RESEARCH**DIRECT DETECTION OF MIRNAS MIR-34A, -145, AND -218 WITH CRISPR/CAS13A-NUCLEASE***O.S. Timoshenko*, L.K. Kurbatov, S.A. Khmeleva, K.G. Ptitsyn, S.P. Radko, A.V. Lisitsa*

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Using CRISPR/Cas13a-nuclease we have demonstrated a feasibility of direct detection of three miRNAs, miR-34a, -145, and -218 (their molecular signature is suggested as a diagnostic and prognostic biomarker in cervical cancer). The detection is based on registration of a cleavage of molecular reporters bearing a fluorophore and a quencher by the complex of CRISPR/Cas13a-nuclease and guide RNA (gRNA) with a spacer of 21-23 nucleotides long. The detection sensitivity varied among miRNAs tested by 10-fold, presumably due to the unwanted intramolecular partial base pairing of gRNA. The miRNA detection with Cas13a nuclease strongly depended on the presence of background RNA thus potentially compromising its direct application to complex media in a general case. Further optimization of measurement conditions including probably an additional amplification of the signal generated by collateral activity of Cas13a nuclease is necessary to directly detect miR-34a, -145, and -218 in biological samples.

Key words: microRNA; detection; CRISPR/Cas-nuclease**DOI:** 10.18097/BMCRM00203**INTRODUCTION**

Small non-coding RNA molecules known as microRNAs (miRNAs) play an important role in posttranscriptional regulation of gene expression [1, 2]. A miRNA is transcribed as a primary microRNA in the nucleus and exported to the cytoplasm as a precursor miRNA where it is processed into a mature miRNA of 18 to 25 nucleotide (nt) long [3]. Aberrations in miRNA expression were shown to closely associate with various diseases including cancer [2, 3]. Due to involvement of miRNAs in cellular regulatory processes, they are considered as promising biomarkers of pathological states, with a focus on detection of cell-free miRNAs circulating in body fluids [4, 5].

The common approach to detection of circulating miRNAs relies on polymerase chain reaction (PCR), using a combination of stem-loop and conventional primers [4, 6]. While PCR is performed in specialized laboratories and requires expensive equipment, presently there is a worldwide call for easy-to-use technologies, which can bring diagnostics closer to a final user – the format known as “point-of-care testing” (POCT) [7]. The emerging CRISPR-based molecular diagnostics relying on the use of CRISPR/Cas-nucleases *per se* or in combination with isothermal amplification methods promises the rapid,

sensitive, and inexpensive detection of nucleic acids and appears to well suit the POCT requirements [8].

Among CRISPR/Cas-nucleases, Cas13a nuclease (further referred to as Cas13a for simplicity) forms a complex with the RNA molecule of a specific sequence – the so-called “guide RNA” (gRNA). The sequence of gRNA is composed of a part responsible for interaction with Cas13a (“direct repeat”) and a part (28 nt long “spacer”) complementary to a target sequence (“protospacer”) [9]. Cas13a activates only if an almost perfect heteroduplex between spacer and protospacer is formed, resulting in a nicking of a target sequence. Upon activation, Cas13a additionally acquires the so called “collateral” or *trans*-activity, which is an ability of the nuclease to unselectively cleave, alongside with the target RNA, all RNA molecules present around [10].

Recently, Shan et al. [11] demonstrated that a miRNA could be directly detected by using complexes of Cas13a and gRNA with a spacer truncated down to 20 nt. In this work, we tested whether this approach can be applied to directly quantify three particular miRNAs, *viz.* miR-34a, miR-145, and miR-218. These miRNAs are shown to be involved in tumorigenesis of cervical cancer [12] and their molecular signature was suggested as a potential diagnostic and prognostic biomarker for this type of cancer [5].



Table 1. Sequences of DNA oligonucleotides used as templates for the enzymatic synthesis of miRNAs and gRNAs

Name	Sequence (5' → 3')
miR145-T	agggattcctgggaaaactggaccctatagtgagtcgtatta
miR145-G	<u>gtccagtttcccaggaatccctgtttagtccccttctgttttggggtagtctaaatccctatagtgagtcgtatta</u>
miR218-T	acatggttagatcaagcacaaccctatagtgagtcgtatta
miR218-G	<u>ttgtgcttgatctaaccatgtgttttagtccccttctgttttggggtagtctaaatccctatagtgagtcgtatta</u>
miR34a-T	acaaccagctaagacaactgccaccctatagtgagtcgtatta
miR34a-G	<u>tggcagtgtcttagctggttgttttagtccccttctgttttggggtagtctaaatccctatagtgagtcgtatta</u>
T7F	taatacgactcactataggg

Note. The sequence of T7 RNA polymerase promoter is highlighted in bold, the sequences of gRNA spacers are underlined.

MATERIALS AND METHODS

Reagents

All chemicals were purchased from “Merck” (USA), if not stated otherwise, and were of the ACS grade or higher. Solutions used were prepared with Milli-Q quality water (18 MΩ·cm). DNA oligonucleotides listed in Table 1 were chemically synthesized and purified by “Evrogen” (Russia). The recombinant Cas13a nuclease was expressed, purified, and characterized as described in details elsewhere [13, 14].

Synthesis of miRNAs and guide RNAs

To synthesize miRNAs and gRNAs, DNA templates (Table 1) were equimolarly mixed with DNA oligonucleotide T7F (Table 1). Its sequence is complimentary to that of the promoter of T7 RNA polymerase (T7 promoter) and annealed by incubating the mixture at 95°C for 2 min, followed by cooling down to room temperature. The gRNA was synthesized using a TranscriptAid T7 High Yield Transcription Kit (“Thermo Fisher Scientific”, USA) according to the manufacturer instructions and purified by phenol/chloroform extraction followed by ethanol precipitation. The RNA pellets were air-dried and dissolved in nuclease-free water. RNA concentrations were measured on a NanoDrop 1000 spectrophotometer (“Thermo Fisher Scientific”). The lengths of synthesized RNAs met those expected as revealed by denaturing gel electrophoresis (data not shown). The aliquots of miRNAs and gRNAs were stored at -80°C until use.

Isolation of total and small RNA from cultured cells

The total RNA was isolated from XL1 Blue strain of *E. coli* with ExtractRNA reagent (“Evrogen”, Russia) and from HepG2 cultured cells with RNeasy

Mini Kit (“Qiagen”, Germany) according to the manufacturers’ protocols. Small RNA (RNA of length < 200 nt) was prepared from the total RNA extracted from HepG2 cells with RNA Clean & Concentrator-25 Kit (“Zymo Research”, USA) following the manufacturer’s instruction. RNA concentrations were determined spectrophotometrically on a NanoDrop 1000 spectrophotometer. Aliquots of total and small RNA preparations in nuclease-free water were stored at -80°C until use.

Measurement of collateral activity of Cas13a ribonuclease

To measure the Cas13a collateral activity, RNaseAlert® Lab Test Kit v2 (“Thermo Fisher Scientific”) was used. The kit includes reporters which are short RNA molecules labelled with a fluorescent dye (presumably fluorescein) and a quencher (further referred to as FQ-reporters). Upon cleavage of a FQ-reporter, the fluorophore and the quencher separate; this results in an increase of fluorescence. The sample preparation was carried out as described earlier [14], with gRNA/Cas13a complexes formed at a molar ratio of 4:1. The TECAN Infinite 200 Pro plate reader (“TECAN”, Austria) was used to monitor fluorescence at 490 nm and 520 nm wavelengths for excitation and emission, respectively. Each 50-μL sample contained 650 ng of Cas13a and varying amounts of miRNAs tested in the absence or presence of either total or small RNA as a background.

RESULTS AND DISCUSSION

Figure 1 shows the kinetics of FQ-reporter cleavage by Cas13a in the presence and absence of target miRNAs. As seen, despite the truncation of a gRNA spacer length down to 21-23 nt, the gRNA/Cas13a complexes are indeed able to recognize targets and acquire the collateral activity. The values

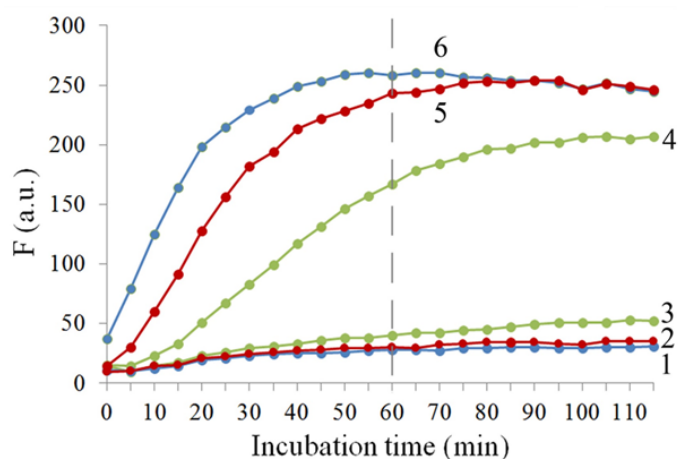


Figure 1. The representative kinetic curves of a cleavage of FQ-reporters by Cas13a nuclease: the fluorescence intensity (in arbitrary units, a.u.) in the absence (curves 1, 2, and 3) and the presence (curves 4, 5, and 6) of miRNAs is shown at every measurement time point. Cas13a in the complex with gRNA targeting miR-145 (curves 1 and 4), miR-218 (curves 2 and 5), and miR-34a (curves 3 and 6). 30 ng of miRNA per reaction.

of fluorescence at 1 h incubation time where they reached or approached a plateau (Fig. 1) were chosen to further evaluate the detection of a target miRNA by a gRNA/Cas13a complex.

Figure 2 demonstrates that Cas13a in the complex with the truncated gRNA does selectively recognize the corresponding target miRNA in the presence of two other miRNAs tested. The dependences of FQ-reporter cleavage on the amount of target present in the reaction mixture for each target miRNAs are shown in Figure 3.

For two miRNAs, miR-218 and -34a, the dependencies were quite similar, with a fast increase and a further saturation at approximately 30 ng (corresponding to 4 pmol) of a target miRNA in the test sample. For miR-145, the fluorescence increase is also saturated at 30 ng of the target but at a substantially lower level (Figure 3). The low limit of detection (LOD) for miR-218 and miR-34a was estimated as ~1 ng per reaction (140 fmol per reaction = 80 nM in the reaction mixture), based on a signal-to-noise ratio of 3, while for miR-145 it was about 10 ng (1.4 pmol).

The LODs for miR-218 and miR-34a are much lower than LOD for miR-145 though the latter is the longest miRNA out of three tested (21 and 22 nt vs. 23 nt, Table 1). Also, stability of the target/spacer heteroduplex (which can be affected not only by the oligonucleotide length but also by its sequence) was the highest for miR-145 among miRNAs tested, as estimated by calculating values of free energy with the RNAcifold tool available at <http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAcifold.cgi> ($\Delta G = -44.63$, -34.64 , and -42.08 kcal/mol for miR-145, -218, and -34a, respectively). Thus, the observed differences

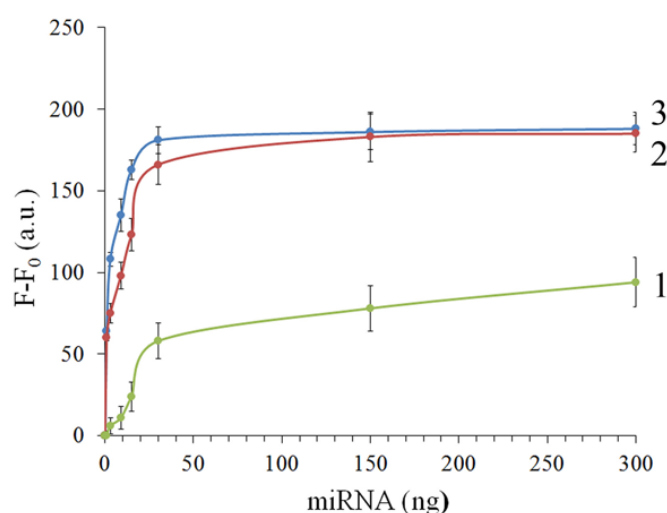


Figure 2. Detection selectivity. The difference between fluorescence in the presence (F) and the absence (F_0) of miRNA at 1 h incubation time for various combinations of miRNAs. Cas13a in the complex with gRNA targeting miR-34a (1), miR-218 (2), and miR-145 (3). Blue bars – the mixture of three miRNAs; red bars – the mixture of two miRNAs not matching the gRNA. 30 ng of each miRNA per reaction tube. The mean values and the corresponding standard errors for three independent measurements are shown.

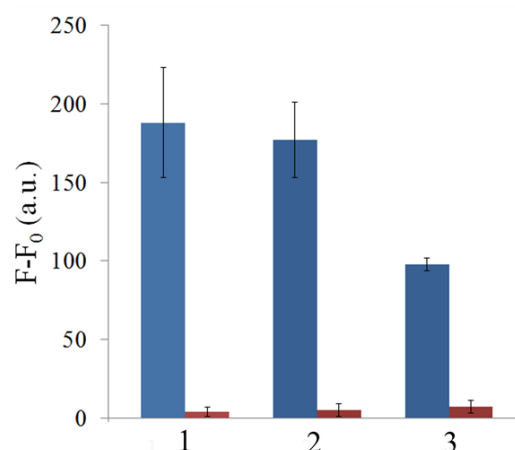


Figure 3. Detection sensitivity. The difference between fluorescence in the presence (F) and the absence (F_0) of miRNA at 1 h incubation time as a function of miRNA amount in the reaction tube. Curves 1, 2, and 3 – miR-145, miR-218, and miR-34a, respectively. Lines are drawn to guide the eye. The mean values and the corresponding standard errors for three independent measurements are shown.

cannot be accounted for by simply assuming that the recognition of miR-145 by the corresponding truncated gRNA is less effective due to pure physical reasons. Furthermore, the best LOD value we obtained (140 fmol) was dramatically higher than that of 5 amol (that corresponded to 0.5-pM target concentration in the reaction mixture) reported by Shan *et al.* for the direct detection of miR-17 (23 nt long) [11]. To some extent, such dramatic differences can be explained by the fact that we performed our experiments under rather suboptimal conditions and a better detection sensitivity can be achieved via their optimization. Yet, it seems doubtful that the optimization could improve the sensitivity by more than 4 orders of magnitude.

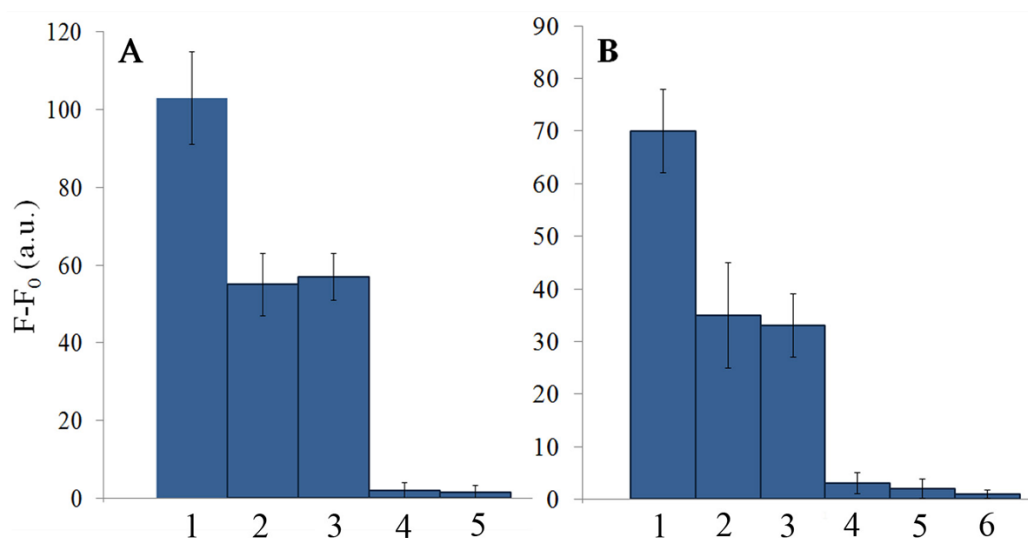


Figure 4. The impact of background RNA on a collateral activity of gRNA/Cas13a complex targeting miR-34a (A) or miR-218 (B). The difference between fluorescence in the presence (F) and the absence (F₀) of RNA at 1 h incubation time for various combinations of miRNAs and total RNA. 1 – miRNA only, 2, 3, and 6 – miRNA in the presence of *E. coli* total RNA, HepG2 total RNA, and small RNA from HepG2 cells, respectively. 4 and 5 – only total RNA from *E. coli* and HepG2 cells, respectively. The amount of miRNA – 3 ng per reaction tube, total and small RNAs – 100 ng per reaction tube.

Besides that, under identical conditions, miR-34a, -218, and -145 demonstrated a 10-fold difference in the detection sensitivity. One cannot rule out that for miR-34a, -218, and -145 the sensitivity, even at optimal conditions, would remain much lower than that reported by Shan *et al.* for miR-17 [11]. It is thought that different spacer sequences can somehow influence an acquiring of collateral activity by Cas13a, for example, by affecting the formation of a gRNA/Cas13a complex via unwanted partial pairing of the spacer with the gRNA repeat sequence and therefore decreasing the actual number of complexes.

To evaluate how the Cas13a-based detection of a target miRNA would be affected by the presence of a large variety of RNA sequences, we have added 100 ng of total RNA either from bacterial (*E. coli*) or from mammalian (HepG2) cells to the reaction mixture containing 3 ng of miR-34a (Fig. 4A) or miR-218 (Fig. 4B).

As seen, in both cases, the registered fluorescence signal decreased by approximately twice but still allowed to reliably detect the presence of target miRNA in the mixture. Interestingly, neither *E. coli* nor HepG2 total RNA *per se* produced appreciable fluorescence signals (Figure 4) thus confirming a high selectivity of gRNA/Cas13a complexes despite the gRNA truncation. However, after addition of 100 ng of small RNAs from HepG2 cells to the reaction mixture containing 3 ng of miRNA-218, no fluorescence signal was observed (Figure 4B). The diversity of RNA sequences is much higher in a preparation of small RNAs due to domination of ribosomal RNA (with a rather limited sequence variety) in total RNA. One

may assume that short-living but multiple interactions of target RNAs and the spacers of corresponding gRNAs with sequences of the background RNA may be attributed to the observed decline in the detection effectiveness due to a partial base pairing. To some extent, this reminds the off-target effects for the case of small interfering RNAs (siRNAs) that is also a result of partial base pairing (*e.g.*, [15, 16]). While an almost perfect base pairing of the gRNA spacer to activate Cas13a, the partial base pairing of gRNA spacer with surrounding RNA sequences can interfere with such pairing, leading to a decrease in an effective concentration of gRNA/Cas13a complexes. Moreover, the partial pairing of a target miRNA with the surrounding RNA sequences can also decrease its effective concentration by the same token. In overall, this can make the direct Cas13a-based detection of miRNAs in a complex medium the additionally sequence-dependent, providing acceptable sensitivity for ones [11] but not for other miRNAs.

Alongside with direct detection of miRNAs with Cas13a, using the simple technique based on a cleavage of FQ-reporters to register the Cas13a collateral activity as in [11] and the present study, more sophisticated approaches were suggested where the additional signal amplification was employed. These approaches include the enhancement of the reporter cleavage signal by means of glucose oxidase mediated conversion of glucose with hydrogen peroxide formation [17, 18], by isothermal amplification methods such as catalytic hairpin assembly (CHA) [19] and exponential amplification reaction (EXPAR)

[20], or by combining the reporter cleavage by Cas13a with subsequent activation of CRISPR/Cas-nuclease Cas12a or Cas14a [21, 22]. In the most cases, the outstanding values of LOD ranged from few zmol to amol per reaction were reported. Apparently, so high sensitivity can alleviate the problem of partial base pairing resulting in a decrease of the effective target concentration and potentially provide the detection of miRNA of any sequence in a complex medium.

CONCLUSION

In this study we have demonstrated the feasibility of direct detection of three miRNAs, miR-34a, -145, and -218. It is based on the registration of a cleavage of molecular reporters bearing a fluorophore and a quencher catalyzed by CRISPR/Cas13a-nuclease activated by the presence of a target miRNA. Among the miRNAs studied tested the detection sensitivity varied by 10-fold, presumably due to the unwanted intramolecular partial base pairing of gRNA. The miRNA detection with Cas13a nuclease strongly depended on the presence of background RNA thus potentially compromising its direct application to complex media in a general case. Further optimization of measurement conditions including additional amplification of the signal generated by collateral activity of Cas13a nuclease is necessary for direct detection of miR-34a, -145, and -218 in biological samples.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or using animals as objects.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Cai, Y., Yu, X., Hu, S., Yu, J. (2009) A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinformatics*, **7**(4), 147-154. DOI: 10.1016/S1672-0229(08)60044-3.
- Saliminejad, K., Khorram Khorshid, H.R., Soleymani Fard, S., Ghaffari, S.H. (2019) An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *J Cell Physiol.*, **234**(5), 5451-5465. DOI: 10.1002/jcp.27486
- Macfarlane, L.A., Murphy, P.R. (2010) MicroRNA: Biogenesis, Function and Role in Cancer. *Curr Genomics.*, **11**(7), 537-561. DOI: 10.2174/138920210793175895
- de Planell-Saguer, M., Rodicio, M.C. (2013) Detection methods for microRNAs in clinic practice *Clin Biochem.* **46**(10-11), 869-878. DOI: 10.1016/j.clinbiochem.2013.02.017
- Aftab, M., Poojary, S.S., Seshan, V., Kumar, S., Agarwal, P., Tandon, S., Zutshi, V., Das, B.C. (2021) Urine miRNA signature as a potential non-invasive diagnostic and prognostic biomarker in cervical cancer. *Sci Rep.*, **11**(1), 10323. DOI: 10.1038/s41598-021-89388-w
- Kiseleva, Y.Y., Ptitsyn, K.G., Radko, S.P., Zgoda, V.G., Archakov, A.I. (2016) Digital droplet PCR - a prospective technological approach to quantitative profiling of microRNA. *Biomeditsinskaya Khimiya.* **62**(4), 403-410. DOI: 10.18097/PBMC20166204403
- Ferreira, C.E.S., Guerra, J.C.C., Shlessarenko, N., Scartezini, M., Franca, C.N., Colombini, M.P., Berlitz, F., Machado, A.M.O., Campana, G.A., Faulhaber, A.C.L., Galoro, C.A., Dias, C.M., Scholnik, W., Martino, M.D.V., Cesar, K.R., Sumita, N.M., Mendes, M.E., Faulhaber, M.H.W., Pinho, J.R.R., Barbosa, I.V., Batista, M.C., Khawali, C., Pariz V.M., Andriolo, A. (2018) Point-of-Care Testing: General Aspects. *Clin Lab.*, **64**(1), 1-9. DOI: 10.7754/Clin.Lab.2017.170730
- Kaminski, M.M., Abudayyeh, O.O., Gootenberg, J.S., Zhang, F., Collins, J.J. (2021) CRISPR-based diagnostics. *Nat Biomed Eng.*, **5**(7), 643-656. DOI: 10.1038/s41551-021-00760-7
- Wu, W.Y., Lebbink, J.H.G., Kanaar, R., Geijsen, N., van der Oost (2018) Genome editing by natural and engineered CRISPR-associated nucleases. *J. Nat Chem Biol.* **14**(7), 642-651. DOI: 10.1038/s41589-018-0080-x
- Abudayyeh, O.O., Gootenberg, J.S., Konermann, S., Joung, J., Slaymaker, I.M., Cox, D.B., Shmakov, S., Makarova, K.S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E.S., Koonin, E.V., Zhang, F. (2016) C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, **353**(6299), aaf5573. DOI: 10.1126/science.aaf5573
- Shan, Y., Zhou, X., Huang, R., Xing, D. (2019) High-Fidelity and Rapid Quantification of miRNA Combining crRNA Programmability and CRISPR/Cas13a trans-Cleavage Activity. *Anal Chem.*, **91**(8), 5278-5285. DOI: 10.1021/acs.analchem.9b00073
- Wang, J.Y., Chen, L.J. (2019) The role of miRNAs in the invasion and metastasis of cervical cancer. *Biosci Rep.*, **39**(3), BSR20181377. DOI: 10.1042/BSR20181377
- Kurbatov, L.K., Radko, P. S., Kravchenko, S.V., Kiseleva, O.I., Durmanov, N.D., Lisitsa, A.V. (2020) Single Stage Purification of CRISPR/Cas13a Nuclease by Metal-Chelating Chromatography Following Heterologous Expression with Preservation of Collateral Ribonuclease Activity. *Applied Biochemistry and Microbiology*, **56**(6), 671-677 DOI: 10.1134/S0003683820060071
- Kurbatov, L., Radko, S., Khmeleva, S., Timoshenko, O., & Lisitsa, A. (2022). Standardization of Recombinant CRISPR/Cas13a-nuclease Preparations by Using RNase A of Known Activity. *Biomedical Chemistry: Research and Methods*, **5**(4), e00177. DOI: 10.18097/bmcrm00177
- Gu, S., Zhang, Y., Jin, L., Huang, Y., Zhang, F., Bassik, M.C., Kampmann, M., Kay, M.A. (2014) Weak base pairing in both seed and 3' regions reduces RNAi off-targets and enhances si/shRNA designs. *Nucleic Acids Res.*, **42**(19), 12169-12176. DOI: 10.1093/nar/gku854
- Seok, H., Lee, H., Jang, E.S., Chi, S.W. (2018) Evaluation and control of off-target repression for RNA interference. *Cell Mol Life Sci.*, **75**(5), 797-814. DOI: 10.1007/s00018-017-2656-0
- Bruch, R., Baaske, J., Chatelle, C., Meirich, M., Madlener, S., Weber W., Dincer, C., Urban, G.A. (2019) CRISPR/Cas13a-Powered Electrochemical Microfluidic Biosensor for Nucleic Acid Amplification-Free miRNA Diagnostics. *Adv. Mater.*, **31**(51), e1905311. DOI: 10.1002/adma.201905311
- Bruch, R., Johnston, M., Kling, A., Mattmüller, T., Baaske, J., Partel, S., Madlener, S., Weber, W., Urban, G.A., Dincer, C. (2021) CRISPR-powered electrochemical microfluidic multiplexed biosensor for target amplification-free miRNA diagnostics. *Biosens Bioelectron.*, **177**, 112887. DOI: 10.1016/j.bios.2020.112887
- Cui, Y., Fan, S., Yuan, Z., Song, M., Hu, J., Qian, D., Zhen, D., Li, J., Zhu, B. (2021) Ultrasensitive electrochemical assay for microRNA-21 based on CRISPR/Cas13a-assisted catalytic hairpin assembly. *Talanta*, **224**, 121878. DOI: 10.1016/j.talanta.2020.121878
- Zhou, T., Huang, R., Huang, M., Shen, J., Shan, Y., Xing, D. (2020) CRISPR/Cas13a Powered Portable Electrochemiluminescence Chip for Ultrasensitive and Specific MiRNA Detection. *Adv Sci (Weinh)*, **7**(13), 1903661. DOI: 10.1002/advs.201903661
- Sha, Y., Huang, R., Huang, M., Yue, H., Shan, Y., Hu, J., Xing, D. (2021) Cascade CRISPR/cas enables amplification-free microRNA sensing with fm-sensitivity and single-base-specificity. *Chem Commun (Camb)*, **57**(2), 247-250. DOI: 10.1039/d0cc06412b
- Zhao, D., Tang, J., Tan, Q., Xie, X., Zhao, X., Xing, D. (2023) CRISPR/Cas13a-triggered Cas12a biosensing method for ultrasensitive and specific miRNA detection. *Talanta*, **260**, 124582. DOI: 10.1016/j.talanta.2023.124582

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ПРЯМАЯ ДЕТЕКЦИЯ МИКРОРНК miR-34a, -145 И -218 С ПОМОЩЬЮ CRISPR/CAS13A-НУКЛЕАЗЫ

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Показана возможность прямой детекции трех микроРНК, miR-34a, -145 и -218 (чья молекулярная сигнатура предлагается как диагностический и прогностический биомаркер при раке шейки матки), с помощью CRISPR/Cas13a-нуклеазы. Детекция основано на регистрации расщепления молекулярных «репортеров» – коротких РНК-олигонуклеотидов, несущих флуорофор и гаситель – комплексом CRISPR/Cas13a-нуклеазы и направляющей РНК (нРНК) со спейсером длиной 21-23 нуклеотида. Чувствительность обнаружения варьировала 10-кратно среди тестируемых микроРНК, предположительно из-за нежелательного внутримолекулярного частичного спаривания оснований нРНК. Обнаружено, что детекция микроРНК с помощью нуклеазы Cas13a сильно зависит от присутствия фоновой РНК, что в общем случае может затруднить такую детекцию в сложном матриксе. Дальнейшая оптимизация условий измерения, включая, вероятно, дополнительное усиление сигнала, генерируемого коллатеральной активностью нуклеазы Cas13a, необходима для прямой детекции miR-34a, -145 и -218 в биологических образцах.

Ключевые слова: микроРНК; детекция; CRISPR/Cas-нуклеаза

ФИНАНСИРОВАНИЕ

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