MiR-378a-3p as a Potential Marker of Xenon Abuse Detection in Blood Plasma during Doping Control

Irina Pronina [1, 2], Elena Mochalova [2] and Pavel Postnikov [2]

[1]Institute of General Pathology and Pathophysiology, Moscow, Russia

[2]National Antidoping Laboratory, Moscow State University, Moscow, Russia

Introduction. The inert gas xenon (Xe) is used in medicine as a safe and high-quality anesthetic during complex surgical interventions, as well as an antipsychotic agent. Sports medicine publications have described the properties of xenon in the recovery processes after prolonged physical training when used in xenon/oxygen (Xe/O2) inhalations. Xenon increases the production of the hormone erythropoietin (EPO) [1] and, as a result, improves the oxygen capacity of the blood, giving a competitive advantage to the athlete in cyclic sports. Consequently, since 2014, xenon is included in the World Anti-Doping Agency Prohibited List, Article S2 “Peptide hormones, growth factors, similar substances and mimetics” paragraph 1.2 “Hypoxia-inducible factor (HIF) activators” (https://www.wada-ama.org/en/prohibited-list). MicroRNAs involved in the regulation of the HIF-signaling can be the markers to indirect determination of the abuse of HIF-activators.

The **aim** of our work was to identify potential marker microRNAs, the alteration in expression of which could reveal the abuse of Xe in cases where the direct determination of this substance becomes practically impossible, for example, after the expiration of Xe excretion from the body.

Methods. Clinical blood test were carried out on analyzer SysmexXN-1000 (Germany). Isolation of miRNAs from blood plasma samples was performed using the PAXgene Blood miRNA Kit (Qiagen, CA, USA). MicroRNA concentrations were measured on an Allsheng Fluo-100B fluorometer (China) using QuDye ssDNA Assay Kit (Lumiprobe RUS Ltd, Russia). RT-qPCR was performed using miRCURY LNA SYBR Green PCR Kits and panels for studying the expression profiles of mature microRNAs of miRCURY LNA miRNA Focus PCR Panel (Qiagen, CA, USA). Reference genes are included in the used panel. Statistical processing of the results was carried out using Bio-Rad CFX Maestro 3.0 software (USA) and online software GeneGlobe (Qiagen, Germany).

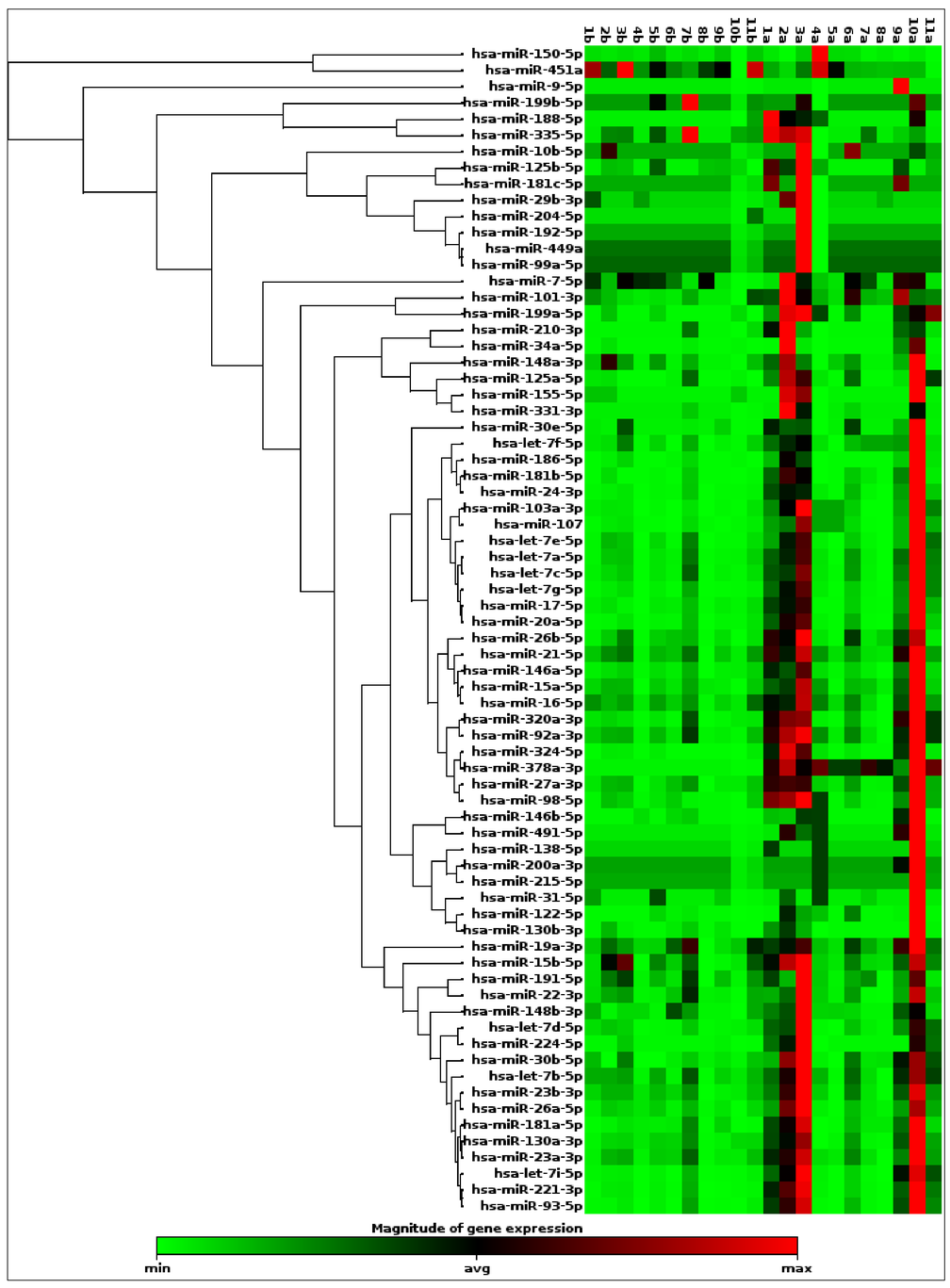


Figure 1. MicroRNAs expression profiles before (b) and after (a) Xe inhalations.

Table 1. Blood parameters before and after Xe inhalations.

	Before Xe/O_2 inhalation course				After Xe/O_2 inhalation course			
	HGB, g/l	HCT, %	RET, %	RBC, 10^{12}/l	HGB, g/l	HCT, %	RET, %	RBC, 10^{12}/l
1	165	46.1	1.81	5.59	169	49.4	2.09	5.76
2	148	41.9	1.44	4.82	157	45.6	1.84	5.11
3	149	42.3	1.66	5.02	158	45.6	1.79	5.38
4	122	34.7	1.10	3.95	125	36.9	1.24	4.08
5	157	43.6	1.51	4.80	161	45.0	1.62	5.02
6	158	43.8	1.16	4.87	162	46.3	1.00	5.07
7	134	36.8	1.24	4.77	138	37.0	1.28	4.81
8	142	36.4	1.15	5.10	144	36.7	1.16	5.15
9	160	44.3	1.48	5.93	165	45.5	1.56	6.02
10	124	36.3	1.32	5.54	129	37.6	1.41	5.56
11	128	36.7	1.35	5.55	128	36.9	1.36	5.61

Results. After five daily Xe/O2 (25:75 v/v) inhalations for 30 minutes, the activation of erythropoiesis, including an increase in the number of erythrocytes, an increase in hemoglobin concentration, etc., up to 5% (Table 1) was determined. Simultaneously, the alterations in the expression profile of microRNAs circulating in blood plasma were observed (Figure 1). 84 microRNAs were analyzed. We found that the expression of hsa-miR-378a-3p in the blood plasma of volunteers after Xe/O2 inhalation increases approximately 70 times ($p<0.05$) and is not determined at all in some volunteers before the inhalations (Figure 2, Figure 3). In addition, the publications provide information about the connection of this microRNA with the pathological processes leading to hypoxia [2].

Figure 2. Volcano plot of mean microRNA expression between samples.

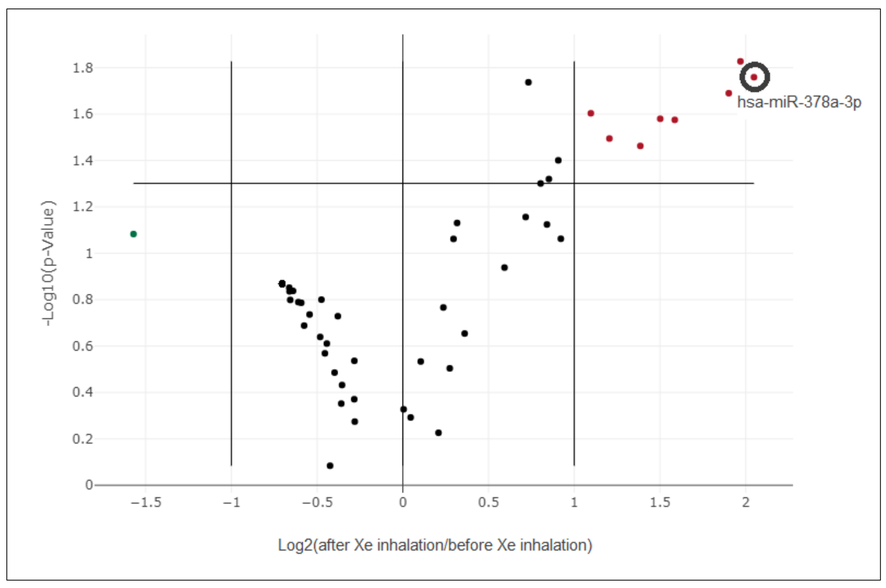
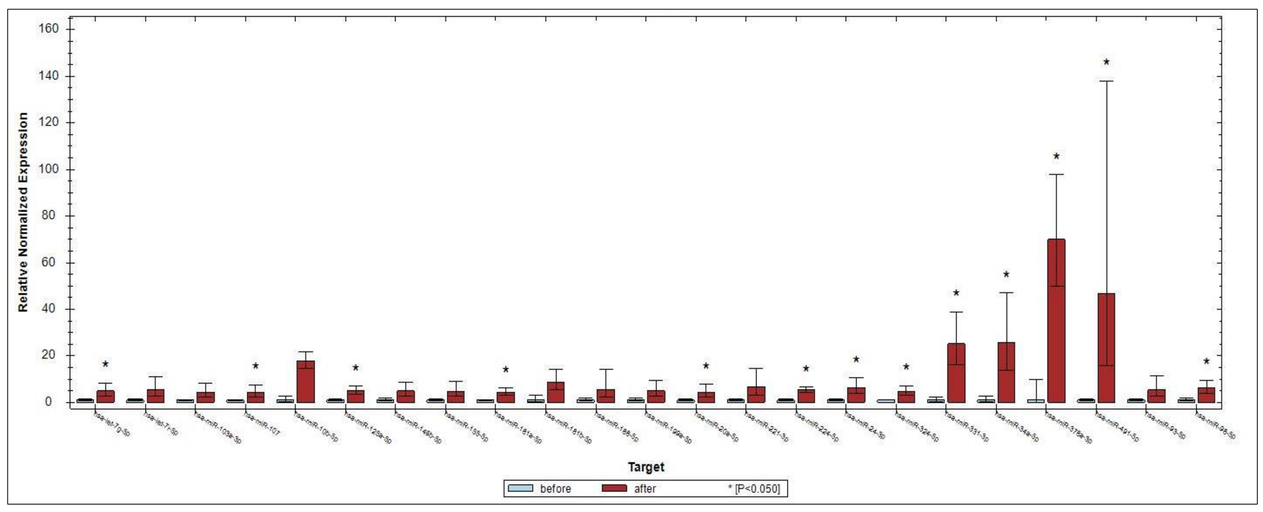


Figure 3. Bar chat representing microRNAs that change their expression to the greatest extent during xenon inhalation.



Conclusion. Thus, hsa-miR-378a-3p can be recommended as a potential candidate for the role of a marker of abuse of Xe in doping control.

Keywords: hypoxia; xenon; microRNA; doping control.

References:

1. Dias KA, Lawley JS, Gatterer H, Howden EJ, Sarma S, Cornwell WK 3rd, Hearon CM Jr, Samels M, Everding B, Liang AS, Hendrix M, Piper T, Thevis M, Bruick RK, Levine BD. Effect of acute and chronic xenon inhalation on erythropoietin, hematological parameters, and athletic performance. J Appl Physiol (1985). 2019 Dec 1;127(6):1503-1510. doi: 10.1152/japplphysiol.00289.2019. Epub 2019 Aug 15. PMID: 31414957.
2. Chen SC, Chen FW, Hsu YL, Kuo PL. Systematic Analysis of Transcriptomic Profile of Renal Cell Carcinoma under Long-Term Hypoxia Using Next-Generation Sequencing and Bioinformatics. Int J Mol Sci. 2017 Dec 7;18(12):2657. doi: 10.3390/ijms18122657. PMID: 29215599; PMCID: PMC5751259.