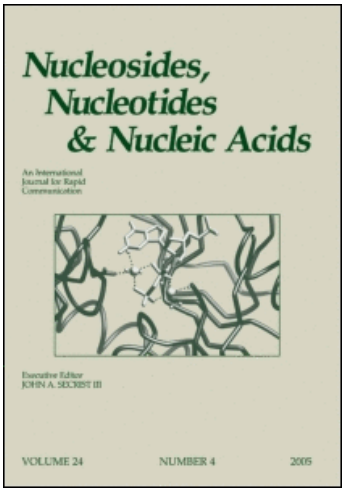

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[a] Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia [b] National Novosibirsk Regional Oncologic Dispensary, Novosibirsk, Russia [c] Central Clinical Hospital of Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia [d] Tomsk Regional Oncologic Dispensary, Tomsk, Russia [e] Scientific Research Institute of Oncology, Tomsk, Russia

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Extracellular Circulating Nucleic Acids in Human Plasma in Health and Disease

Pavel P. Laktionov,[1,*] Svetlana N. Tamkovich,[1] Elena Yu. Rykova,[1] Olga E. Bryzgunova,[1] Andrey V. Starikov,[2] Nina P. Kuznetsova,[2] Sergey V. Sumarokov,[3] Sergey A. Kolomiets,[4] Natalya V. Sevostianova,[5] and Valentin V. Vlassov[1]

[1]Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia
[2]National Novosibirsk Regional Oncologic Dispensary, Novosibirsk, Russia
[3]Central Clinical Hospital of Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia
[4]Tomsk Regional Oncologic Dispensary, Tomsk, Russia
[5]Scientific Research Institute of Oncology, Tomsk, Russia

ABSTRACT

The concentration of extracellular DNA and RNA in blood plasma of healthy donors, trauma patients, patients with breast and lung cancer, nonmalignant breast tumors and nonmalignant lung diseases were estimated. Significant amounts of extracellular RNA were found in plasma of trauma patients. The concentration of DNA and RNA in plasma of trauma patients correlates with the extent of posttraumatic organ failure. Extracellular RNA was not found in the plasma of breast cancer patients and patients

*Correspondence: Pavel P. Laktionov, Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, 8, Lavrentiev Ave., Novosibirsk 630090, Russia; E-mail: lakt@niboch.nsc.ru.

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with nonmalignant breast tumors, whereas a very high concentration of extracellular RNA was found in patients with malignant and nonmalignant diseases of lung.

Key Words: Circulating DNA; Circulating RNA; Breast tumors; Trauma; Lung disease.

The concentration of extracellular DNA (exDNA) in blood correlates with some pathologies.[1–4] This cell-free DNA is rather stable in the bloodstream and can circulate in various forms in blood: as naked DNA, associated with histones in nucleosomes, bound to other plasma proteins or packed in apoptotic bodies.[5] The concentration of extracellular RNA (exRNA) considered as a less stable molecule was not determined to date. However, novel data about particle-associated RNA recirculation,[6] stability of exRNA in blood serum and plasma[7] and successful RT-PCR analysis of serum RNA demonstrate the existence of prominent RNA level in human blood.

The objective of this work was to study concentrations of exDNA and exRNA in the plasma of healthy donors and patients with different pathologies.

Blood samples of breast cancer and nonmalignant patients were obtained from the National Novosibirsk Regional Oncologic Dispensary. Blood samples of lung cancer and nonmalignant patients were obtained from Tomsk Regional Oncologic Dispensary. All of the patients were previously untreated. The tumor staging was performed according to the TNM classification. Blood samples of healthy donors were obtained from Novosibirsk Central Clinical Hospital and Novosibirsk Military Hospital. All blood samples (2.4 ml) were collected into tubes containing sterile PBS with 10 mM EDTA (0.6 ml). Plasma was collected after pelleting of the cells by low speed centrifugation. Plasma samples were prepared within 4 hours after taking of the blood. The samples were stored frozen at $-40°C$ in aliquots and were thawn once before investigation. A quantitative method of nucleic acids isolation was used for isolation of nucleic acids (NA) from plasma samples.[8,9] The concentration of NA was measured with a two-step fluorescence-based assay for quantification of RNA and DNA using Hoechst 33258 and SYBR Green II dyes in solutions containing both nucleic acids.[10]

The average concentration of exDNA in plasma of healthy donors was 38 ng/ml and was close to previously described.[3] ExRNA in a concentration of about 250 ng/ml was found in 30% of plasma samples from healthy donors (3 from 9) (Table 1).

Table 1. Extracellular NA in the blood of healthy donors.

Donors	Age	Sex	DNA, ng/ml	RNA, ng/ml
1	23	M	37	256
2	22	M	38	0
3	19	M	39	0
4	20	M	35	291
5	23	M	40	0
6	22	M	36	0
7	22	M	30	0
8	24	M	0	223
9	21	M	53	0

Table 2. Extracellular NA in plasma of patients with trauma.

Patients	Age	Sex	DNA ng/ml	RNA ng/ml	Diagnosis
1	62	F	2283	179	Multiple fractures (N = 4)
2	50	M	3143	1216	
3	16	M	2751	1229	
4	14	M	353	1327	
5	66	F	319	12	Single fracture (N = 4)
6	86	M	251	113	
7	29	F	448	300	
8	35	F	181	119	
9	68	F	54	42	Soft tissue bruise (N = 6)
10	31	M	0	39	
11	38	M	37	100	
12	21	M	41	20	
13	54	M	56	50	
14	63	F	68	10	

Table 3. Extracellular NA in plasma of patients with lung disease.

Patients	Age	Sex	DNA, ng/ml	RNA, ng/ml	Stage	Diagnosis
1	67	M	61	1294	$T_2N_0M_0$	Non-small cell
2	72	M	41	420	$T_2N_0M_0$	lung cancer
3	53	M	35	344	$T_2N_0M_0$	
4	61	M	48	364	$T_2N_0M_0$	
5	45	F	99	388	$T_2N_0M_0$	
6	57	M	173	140	$T_3N_0M_0$	
7	66	M	129	3306	$T_3N_0M_0$	
8	57	M	72	410	$T_3N_0M_0$	
9	69	M	82	1608	$T_3N_0M_0$	Small cell lung cancer
10	61	M	36	1474	$T_3N_1M_0$	Pulmonary adenocarcinoma
11	41	M	50	422		Nonmalignant chronic disease of lung
12	59	M	46	366		
13	41	M	17	374		
14	62	M	46	286		
15	61	M	41	346		
16	62	M	181	356		
17	51	M	167	278		
18	54	M	158	480		
19	60	M	280	94		
20	56	M	1444	328		Carrier of isotopes
21	75	M	0	1642		Pneumonia
22	31	F	61	1682		
23	42	F	0	1708		
24	42	M	164	0		
25	52	M	0	191		
26	46	M	60	1858		

Table 4. Extracellular NA in plasma of patients with breast disease.

Patients	Age	Sex	DNA, ng/ml	RNA, ng/ml	Stage	Diagnosis
1	35	F	308	0	$T_1N_0M_0$	Breast cancer
2	54	F	401	0	$T_1N_0M_0$	
3	38	F	265	0	$T_2N_0M_0$	
4	44	F	225	0	$T_2N_1M_0$	
5	39	F	83	0	$T_2N_1M_0$	
6	49	F	184	0	$T_2N_1M_0$	
7	51	F	875	0	$T_2N_xM_0$	
8	63	F	1414	0	$T_3N_2M_0$	
9	42	F	178	0	$T_3N_2M_0$	
10	66	F	652	0	$T_4{}^5N_1M_0$	
11	54	F	503	0	$T_4{}^5N_1M_0$	
12	13	F	0	0	Fibroadenoma	
13	46	F	121	0	Cystoepithelioma	

The applicability of measuring of plasma DNA concentration for early prediction of posttraumatic organ failure or multiple organ dysfunction syndrome was shown earlier.[1] The applicability of estimation of exRNA concentration for prediction of hidden trauma was tested in a preset investigation. It was shown that the concentration of exDNA and exRNA in the plasma of patients with multiple fractures is significantly higher then those in the plasma of patients with single fractures and soft tissues damages (Table 2) and can probably be used for prediction of hidden trauma.

A high exRNA level was shown in plasma of patients with pneumonia and lung cancer. The level of exRNA does not correlate with cancer stage and may be concerned with the inflammation process. Similar levels of exDNA were found in plasma of cancer patients and patients with chronic nonmalignant lung disease (Table 3). The high level of exRNA in plasma of lung cancer patients provides opportunities for the development of a lung cancer diagnostic based on analysis of cancer specific RNA sequences.

ExRNA was not found in the plasma of patients with breast cancer and nonmalignant breast tumors whereas the level of exDNA in plasma of breast cancer patients noticeably exceeded normal level (Table 4).

A strong decrease in the plasma exRNA of women accompanied with an exDNA increase could be indicative of breast tumor development. The data obtained indicate that the estimation of plasma exNA concentration could be used as a screening assay for the general health check up for men, while more accurate assay for tumor-specific DNA should be developed for cancer diagnostics.

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