Plasma Content of Extracellular Nucleic Acids in Donors and Patients with Mammary Tumors

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The concentrations of extracellular DNA and RNA were measured in the plasma of donors and patients with fibroadenoma and breast cancer. The content of extracellular DNA surpassed the normal in 80% plasma samples from patients with mammary tumors. Extracellular RNA was detected in 30% plasma samples from donors and patients with breast tumors. No correlations were found between plasma concentration of extracellular DNA and size and stage of tumor growth. Hence, measurement of extracellular DNA in the plasma of patients can be used only as an accessory test for tumor diagnosis.

Key Words: *extracellular DNA; extracellular RNA; mammary fibroadenoma; breast cancer*

Breast cancer (BC) is the most prevalent oncological disease in women (more than 20% of the total number of neoplasms) associated with high mortality (more than 60% of patients) [7]. Like for other types of cancer, early diagnosis is an obligatory condition of effective therapy. A perspective approach, which can be used for early noninvasive diagnosis of cancer, are measurements of the blood content of extracellular nucleic acids (NA) and evaluation of their composition.

Free circulating extracellular DNA is present in low concentrations (0-100 ng/ml) in donor blood [8]. The concentration increases to 118±14 ng/ml in benign tumors and to 412±63 ng/ml in malignant tumors [7]. The concentration of circulating DNA does not correlate with tumor location, but serum concentration of DNA is higher in patients with metastases [10]. Measurements of free circulating DNA in the plasma of cancer patients showed that the greater portion of DNA was released by tumor cells [3]. RNA of tumor origin was detected in the plasma of patients with breast cancer, melanoma, and colorectal cancer [1, 4,9]. The concentration of extracellular RNA in health and disease was never measured, which can be due to the absence of sensitive screening methods for selective evaluation of RNA concentrations.

We measured the concentrations of extracellular DNA and RNA in the plasma of donors and patients with breast tumors.

MATERIALS AND METHODS

Blood samples of donors (n=9) and untreated patients with fibroadenoma (n=13) and breast cancer (n=20) were analyzed. The disease stage was determined by the TNM classification. The blood was stabilized with 0.05 M EDTA in phosphate buffered saline (1:5 blood: EDTA ratio), stored at 4°C, and analyzed within 6 h. Blood cells were precipitated by 15-min centrifugation at 450g, the supernatant was collected, and the cells were centrifuged for 15 min at 20,000g. The plasma was collected and stored in aliquots at -20°C.

Extracellular NA were isolated by adsorption on finely dispersed glass. Buffer (2 ml) containing 6.75 M guanidine thiocyanate, 60 mM EDTA, 15 mM Tris-acetate (pH 6.4), and 3 mg fine glass dispersion was

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TABLE 1. Plasma Concentration of Extracellular NA in Donors and Patients with Breast Tumors

Patient	DNA, ng/ml	RNA, ng/ml	Diagnosis	
1	37	256	Donors	
2	38	0		
3	39	0		
4	35	291		
5	40	0		
6	36	0		
7	30	0		
8	20	223		
9	53	0		
10	26	64	Mammary fibroadenoma	
11	82	0		
12	269	74		
13	590	0		
14	0	0		
15	0	0		
16	75	0		
17	352	0		
18	436	0		
19	0	0		
20	580	20		
21	251	0		
22	70	30		
23	512	0	T1N0M0	Breast cancer
24	410	0	T1N0M0	
25	187	0	T1N0M0	
26	0	0	T1N0M0	
27	0	0	T2N0M0	
28	998	0	T2N0M0	
29	36	29	T2N0M0	
30	20	0	T2N1M0	
31	393	0	T2N1M0	
32	182	499	T2N1M0	
33	75	51	T2N2M0	
34	1827	0	T2NXM0	
35	107	181	T2NXM0	
36	98	34	T2NXM0	
37	2160	0	T3N0M0	
38	804	0	T4-5N1M0	
39	1533	0	T4-5N1M0	
40	92	0	T4-5N1M0	
41	60	0	T4-5N1M0	
42	188	0	T4-5NxM0	

Note. NA concentration below the sensitivity threshold of the method is shown as 0.

added to 1 ml sample. After 5-min incubation on a shaker at ambient temperature, 2.7 ml isopropanol and 7.8 ml buffer (40 mM EDTA, 10 mM Tris acetate pH 4.5, and 3 mg finely dispersed glass) were added. After 5-min incubation on the shaker at room temperature the glass suspension was precipitated by 30-sec centrifugation at 1000*g*. The supernatant was discarded, the precipitate was washed twice with a buffer containing 4.5 guanidine thiocyanate, 40 mM EDTA, 10 mM Tris acetate (pH 6.4), and twice in a buffer containing 25% isopropanol, 100 mM NaCl, 10 mM Tris-HCl (pH 8.0). Extracellular NA were eluted from the glass surface on a shaker for 2 min. NA from reference DNA samples (for construction of the calibration curve) and from analyzed samples were isolated in parallel.

The concentration of extracellular DNA in analyzed samples was measured using Hoechst 33258 on a VersaFluor fluorimeter (Bio-Rad). The concentration of extracellular RNA was determined by the difference of NA concentrations evaluated by fluorescence of their complexes with SYBR Green II stain and DNA concentration measured using Hoechst 33258 intercalating stain [5].

RESULTS

The extracellular DNA concentration in donors fits to normal distribution (χ^2). The mean concentration of extracellular DNA in the plasma of donors aged 18-30 years was 34±13 ng/ml (Table 1). Our results are in line with the data of other authors, who measured DNA concentrations by PCR [3,8] and nick translation.

In 30% samples the concentration of extracellular RNA was 256 ng/ml.

The content of extracellular DNA in patients with mammary fibroadenoma surpassed the normal in 69% cases and extracellular RNA was detected in 31% cases. The concentration of extracellular DNA was elevated in 80% patients with breast cancer. Extracellular RNA was detected in 25% cases (Table 1).

The concentration of extracellular DNA in donor plasma differed significantly from DNA concentration in the plasma of patients with breast cancer and patients with benign tumors (p=0.000026 and p=0.01829, respectively, according to Mann—Whitney test). Differences in the concentrations of extracellular DNA in patients with mammary fibroadenoma and breast cancer were negligible.

The proposed method can be used for screening measurements of extracellular NA in the plasma and for detecting patients at risk of cancer. The decrease of the concentration of circulating DNA after effective radio- and chemotherapy suggests that measurement of NA concentrations can be used for monitoring of anticancer therapy.

Low concentration of extracellular RNA in the plasma in breast cancer can be explained by high activity of plasma RNase [4], which limits the use of oncospecific RNA sequences for the diagnosis of breast cancer.

It seems that accurate diagnosis of cancer requires analysis of oncospecific DNA sequences [8], including analysis of methylated oncogenesis genes, oncospecific mutations in protooncogenes [8], or studies of microsatellite DNA [2].

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