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POINT OF VIEW

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COMPARISON OF THE NANO-SIZED PARTICLES NUMBER IN BLOOD PLASMA AND ON THE ERYTHROCYTES SURFACE USING SCANNING ELECTRON MICROSCOPY IN A CERVICAL CANCER PATIENT

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To explain the more productive isolation of HPV DNA from the blood component compared to plasma in cervical cancer patients, using scanning electron microscopy images of venous blood were studied. It was revealed that there are more nanosized bioparticles on the erythrocytes surface than in plasma. It has been suggested that among them there may be tumor extracellular vesicles carrying HPV DNA. To confirm that the erythrocyte fraction of blood is a more productive biological sample for isolating HPV DNA, continued studies are needed.

Keywords: human papillomavirus, screening, extracellular vesicles.

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Despite widespread screening in Russia to prevent cervical cancer, mortality from it remains high and has not decreased significantly. Residents of the regions of the Arctic zone of the Russian Federation (AZRF) feel this to a greater extent [11]. In the territories of the Russian Arctic there are difficulties in solving government tasks to improve the standard of living of the population and provide them with quality goods and services. It is believed that the main reasons are the weather conditions, the size of the territory, insufficient or even absence of transport infrastructure, significant dispersion of settlements, low population density, nomadism, etc. [8].

These reasons also negatively affect the use and effectiveness of traditional methods of screening for cervical pathology in women living in the Arctic. Therefore, there is an urgent need to develop simple tests applicable in hard-to-reach settlements of the Russian Arctic, that

represent an alternative to traditional screening for cervical pathology. In this regard, identifying cervical pathology and cervical cancer using a blood test is, in our opinion, the optimal choice. Indeed, blood sampling is easy, it can be carried out at the patient's location, and one container for blood is needed. While for traditional screening the collection of biomaterial requires an equipped obstetrician-gynecologist's office and a larger number of consumables - a gynecological speculum, a cytobrush, glass slides , vial, etc.

Currently, as early markers of cervical cancer, researchers suggest using some special characteristics of blood identified by such methods as, for example, measuring red cell distribution width (RDW) [14], differential scanning calorimetry [7], Raman spectroscopy [6], etc. Scientific directions searching for the cervical cancer markers in the blood also include studies of the proteome, metabolome, transcriptome and genome [15,20].

In our opinion, the studies on the detection of tumor DNA in the blood are especially promising as a cervical cancer early marker. It is known that the cause of cervical cancer is the human papillomavirus (HPV), and HPV DNA fragments detected in the blood can be attributed to cervical cancer tumor DNA [18]. But tumor DNA circulating in the blood is highly fragmented, its concentration can be extremely low [5], the half-life ranges from 30 minutes to two hours [1]. This explains the complexity and still low sensitivity of cervical cancer detecting tests based on the isolation of circulating tumor DNA in the blood.

Plasma or serum are using as a biological sample to isolate cervical cancer tumor DNA in the vast majority of studies. However, it has been shown that HPV DNA fragments can be detected in the erythrocyte component of blood [10, 12], and in this component they are found more often than in plasma [12]. Now we suppose that the isolation of cervical cancer tumor DNA from the erythrocyte component of blood may be more productive than from whole blood, plasma and serum.

Tumor DNA circulating in the blood may be cargo in extracellular vesicles secreted by tumor cells [4]. One of the methods for studying extracellular vesicles is scanning electron microscopy [9,14]. Biological nanosized particles of endogenous origin were visualized on the surface of erythrocytes in cervical cancer patients by scanning electron microscopy [14]. Among which, probably, there may be tumor extracellular vesicles.

The purpose of this study was to examine cervical cancer patient's blood images using scanning electron microscopy to compare the number of nanosized particles in the blood plasma and on the surface of red blood cells. The presence of a larger number of nanosized particles on the erythrocytes surface than in plasma, albeit indirectly, may explain the more productive isolation of HPV DNA from the erythrocyte component of blood than from plasma.

Materials and methods. Smears for imaging were made from the venous blood of a patient at the Yakut Republican Oncology Center with newly diagnosed cervical cancer. The patient gave written informed consent to the study. Venous blood was collected in the morning, on an empty stomach, into a vacuum blood container with K3-EDTA. A drop of the resulting blood was smeared in a thin even layer on a clean fat-free glass slide and air-dried at room temperature for 24 hours. Blood images were obtained using a high-resolution scanning electron microscope JSM-7800F (JEOL, Japan) by detecting secondary electrons with a lower detector at an accelerating voltage of 1 kV and a focal length of 4.2 millimeters. Glass substrates with blood smears were fixed by using carbon tape. The image magnification of blood samples ranged from 500 to 20,000 times.

Results and discussion. A series of images of venous blood from a patient with cervical cancer were obtained with magnification from 500 to 20,000 times. Clear visualization of nanosized particles became possible with magnifications of 5,000 times or more (Figure 1). The surface area of erythrocytes and the area of blood plasma located between them have approximately equal proportions in the resulting image. Nanosized particles are characterized by a white color; a study of their elemental composition in a previous study showed the content of carbon, nitrogen and oxygen in them, which confirmed their organic origin [14]. In Figure 1, some of the nanosized particles are marked with white arrows, and it can be seen that they are much more common on the surface of red blood cells than in plasma. The particle sizes in the images were approximately 70 nm (Fig. 2), this value is within the size range of extracellular vesicles.

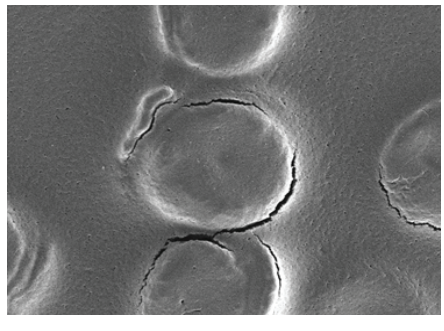

Fig. 1. SEM image of a venous blood sample from a patient with cervical cancer at 5,000x magnification

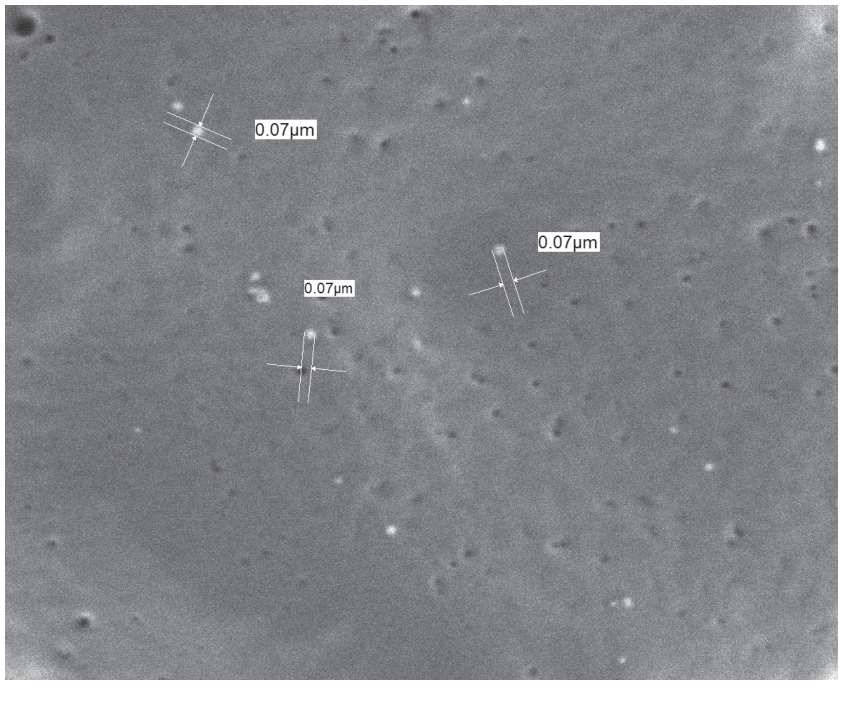

Fig. 2. SEM image of the surface of a red blood cell from the venous blood of a patient with cervical cancer at a magnification of 20,000 times

Extracellular vesicles are secreted by almost all mammalian cells, including cancer cells, and there is high variation in their secretion rates between different cell types. Extracellular vesicles are bounded by a bilipid outer layer and contain a wide range of bioactive molecules that play a critical role in regulating a variety of natural processes in the human body, as well as in pathological processes such as viral infection, the development of cancer and its metastases [4].

It has been established that DNA incorporation into extracellular vesicles is important for maintaining cellular homeostasis; inhibition of their secretion leads to the accumulation of nuclear DNA in the cytosol, provoking a senescence-like phenotype, leading to cell cycle arrest and ultimately to apoptosis. Secretion of DNA through extracellular vesicles protects tumor cells from the inflammatory response. It is assumed, that higher DNA concentrations are associated with more larger sizes of extracellular vesicles, and a greater amount of DNA is contained in extracellular vesicles of tumor cells compared to normal cells. DNA included in extracellular vesicles of tumor cells reflects their genome and is protected from nucleases [4].

Of course, extracellular vesicles observed on the surface of erythrocytes can be secreted by the erythrocytes themselves and adhere to their surface [16]. But it is known that many of the erythrocyte adhesion receptors are similar to those of other cells [19]. Extracellular vesicles also have adhesion receptors, with the help of which they interact with recipient cells [2]. In addition, it has been established that the lipid and protein profiles of erythrocyte membranes in healthy patients and cancer patients differ, and the differences are not associated with the nutritional characteristics of patients [3,17]. That is, the possibility of adhesion of extracellular vesicles, including produced those by tumor cells, on the surface of erythrocytes cannot be ruled out.

We recognize, that the significant predominance of the number of nanosized particles on the surface of erythrocytes compared to blood plasma, which we detected using scanning electron microscopy in the images of the venous blood of the cervical cancer patient, does not directly confirm that the erythrocyte component of blood is a more productive biological sample for isolating cervical cancer tumor DNA. Further molecular genetic studies are needed to establish this. It is necessary to note that modification of the scanning electron microscope with a thermal field Schottky cathode and a superhybrid lens at low accelerating voltages made it possible to study blood samples without applying a conductive coating to them, which distorts the image of surfaces in the nanometer range. Using this modification allowed us to visualize nanosized particles in blood samples and determine their location and size.

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