extraction time is 30 minutes, the precipitating agent is 2% citric acid. , the yield of hyaluronic acid is 4.87-4.89%. We prepared a 1% solution of hyaluronic acid, obtained on the basis of optimal technological parameters, and evaluated its solubility, pH and authenticity. It has been proven that the physicochemical properties of hyaluronic acid obtained from rooster combs in the laboratory are the same as those of a standard preparation. With this in mind, a number of hyaluronic acid-based cosmetics will be developed in the future: creams, gels, lotions and balms.

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Zeta potentials of healthy and cancer cells of human lung: implication to cancer therapy

[1]R.B.Gasanova, [1,2]L.A.Melikova, [1]O.K. Gasymov, [2]J.A. Aliyev
[1] Institute of Biophysics, Azerbaijan National Academy of Sciences, Baku
[2] National Center of Oncology, Azerbaijan Republic Ministry of Health, Baku

Açar sözlər: ağciyər xərçəngi, karsinoma, hüceyrələrin zeta-potensialı, hüceyrəxarici mühit, interstisial maye, nanotəbabət
Ключевые слова: рак легкого, карцинома, зета-потенциалы клеток, внеклеточная среда, интерстициальная жидкость, наномедицина
Keywords: lung cancer, carcinoma, zeta potentials of cells, extracellular environment, interstitial fluid, nanomedicine

Metabolic activities of normal and cancer cells differ significantly from each other and can be assessed by measuring the concentration of low-molecular-weight metabolites. These metabolites represent a diverse range of chemicals, full characterization of which is an impossible task for any single method [1]. In addition, modifications of some functional groups (for example, esterification) have been observed in the body fluid of cancer patients as a result of altered metabolism [2]. Different methods, such as a nuclear magnetic resonance (NMR), mass spectroscopy (MS), Raman and FTIR spectroscopies, have been developed for the cancer diagnostics or screening proposes by utilizing the differences in metabolic activities of cancer and normal cells. An artificial Intelligence-based medical diagnosis was developed using the model constructed with the FTIR spectra of human blood plasma of healthy and lung cancer patients [3].

Changes in metabolism observed in the various cancer types result in modification of morphology of the cells, surface receptors and lipid compositions localized in the plasma membrane, etc [4-6]. During cancer progression, significant changes were observed in the extracellular environments of cancer cells. Increased oxygen consumption in the rapidly growing tumor cells deprives oxygen supply that results in hypoxic microenvironments in cancer cells. In hypoxia, as a result of enhanced anaerobic glycolysis, the extracellular environment of cancer cells becomes more acidic [7]. The pH value of the cancer microenvironment may reach to 6.2. An acidic environment along with the activation of some lysosomal enzymes initiates the expression of some genes involved with pro-metastatic factors. Therefore, the extracellular acidic environment is closely related to cancer metastasis [8]. As mentioned above, metabolic changes that arise in cancer cells modify the plasma membrane including the lipid composition. The surface charge of cancer cells is different compared to that of normal cells. Proper characterization of the surface charges is very important since it is essential to characterize and distinguish various cells. The surface charge of cells can be characterized quantitatively with zeta potential measurements. Zeta potential is an electric potential that characterizes the electrical double-layer potential of the cell surface [9, 10]. In this regard, the zeta potential value of the cells is very informative for the assessment of the stability of the

healthy and cancer cells. Increased values (more negative) of the zeta potential of cells indicate that these cells are less prone to cell aggregation and adhesion processes [11]. Zeta potential measurements were applied to study the interaction between nanoparticles and biological cells. Multifunctional nanoparticles are fabricated for the diagnosis and treatment of cancer cells. This new medical approach is still evolving [12].

In the current study zeta potential measurements were performed for human lung cancer cells, specifically large cell carcinoma, and non-cancer cells (called healthy). Data indicate that zeta potential values of cancer and healthy cells are significantly different. In contrast to pH 6.2, which is relevant to the environment of hypoxic cancer cells, zeta potential values decreased for both types of cells at pH 7.3. The data are valuable for cancer diagnosis as well as for the assessment of drug-cell interaction and drug delivery.

Materials and Methods. *Sample preparation.* Human lung materials from the surgery were examined in a histopathology laboratory for diagnosis and separation of the material to healthy and carcinoma parts. A 62-year-old smoker man had an abnormal pulmonary mass in the upper lobe of the lung in computed tomography (CT) imaging. Histopathology has indicated the presence of poorly differentiated large cell carcinoma. The final diagnosis was T3N2M1b in TNM classification indicating that cancer spread nearby lymph nodes with solitary metastasis.

The lung tissues from the above-indicated patient were obtained after fixation in 10% formalin. Before the experiments, formalin was removed from the lung tissues by placing them PBS buffer at pH 7.3 and six 15-minute washes with the same buffer. The research was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from the donor after the explanation of the objective of the study. The sample handling and research procedures were approved by the institutional review board.

Preparation of the cell suspension. Cancer and adjacent non-cancer (named as healthy) lung tissue specimens were cut into a small species and then homogenized using a glass homogenizer in PBS buffer at pH 6.2 or 7.3. Homogenates, washed several times in appropriate buffer, were centrifuged for 5 minutes.

Zeta potential measurements. The zeta potential measurements were performed for healthy and carcinoma cells of human lung suspended in phosphate-buffered saline (PBS, in mol L^{-1}: NaCl, 0.14, KCl, 0.005, Na_2HPO_4, 0.01, KH_2PO_4, 0.002, pH 7.3), ionic strength of which is 0.177 mol L^{-1}. To evaluate the influence of pH, the pH value of the same dispersing solution was adjusted to 6.2. Zeta potential measurements were performed at a temperature interval of 25- 45 ^oC. To get the correct sample temperature value, the samples were equilibrated at least 10 min at each temperature.

Zeta potentials of carcinoma and healthy cells of the human lung were measured using Zetasizer Nano-ZS (Melvern Panalytical Ltd, UK) in automatic mode to select parameters. For each experiment, 10 to 15 measurements were performed. Disposable zeta cells were used for measurements. Data were recorded and analyzed using a Malvern Zetasizer Software. Zeta potential distribution plots were analyzed using the program OriginLab 2016 (OriginLab. Corporation, Northampton, MA, USA).

Results and Discussion. Figure 1 shows the results of Zeta potential measurements for large-cell carcinoma and healthy cells in pH values of 6.2 and 7.3 at the temperature interval of 25- 40 ^oC. Zeta potential values for cancer and healthy cells are significantly different at both pH values.

At pH 6.2, which is characteristic of the hypoxic cancer environment, Zeta potential values for the healthy and cancer cells lie between about -28 mV ÷ -31 mV and -23 mV ÷ -26 mV, respectively. However, at pH 7.3, which is characteristic of a normal extracellular environment, Zeta potential values for the healthy and cancer cells shifts significantly and lie between about -25 mV ÷ -26 mV and -19 mV ÷ -22 mV, respectively. The differences in Zeta potential values in pH of 6.2 and pH of 7.3 are very interesting. The absolute values of Zeta potentials of the carcinoma cells in pH 6.2 are the lowest (Figure 1). It is well established that Zeta potential is directly related to the surface charges of the cell membrane [11]. Higher values of the Zeta potentials, for example of nanoparticles, cells, etc., indicate the stability of the systems. Thus, alkalization of the microenvironment of the hypoxic cancer cells from pH 6.2 to pH 7.3 will decrease the stability of the cancer cells. The use of alkalizing drugs in chemotherapy may be beneficial for cancer patients.

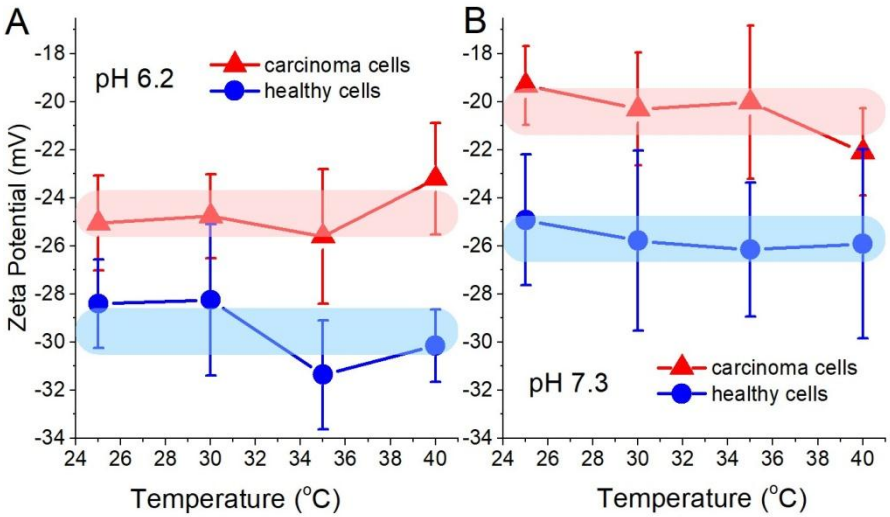

Figure 1. Zeta potentials of human lung healthy and carcinoma cels at various conditions. (A) Zeta potential values of human lung healthy and carcinoma cells in pH 6.2 at temperature interval of 25-45 degree C. (B) Zeta potential values of human lung healthy and carcinoma cells in pH 7.3 at temperature interval of 25- 45 degree C. Thick horizontal lines represents the average value of Zeta potential of corresponding cells.

In cancerous tissue, the cancer cells are heterogeneous and may show different properties [13]. Since the value of Zeta potential related to the surface charges of cell membranes, Zeta potential distribution curves of the healthy and carcinoma cells were examined. It is evident that for the carcinoma cells, amplitude averaged values of the Zeta potential distributions do not coincide with the maximum values in both pH values of 6.2 and 7.3. This indicates that the Zeta potential data do not follow a normal distribution. Therefore, Zeta potential differences in cancer cells are not random, but primarily determined by metabolic differences. However, this situation is not observed for healthy cells. The heterogeneous nature of the carcinoma cells may indicate that the carcinoma cells are in different metabolic states in the cancer tissue. Interestingly, the cancer cells show less heterogeneity in pH value of 7.3, which is a case for the normal extracellular microenvironment.

Indeed, the carcinoma cells in pH 6.2 show the highest FWHM for Zeta potential. In contrast, the FWHM of Zeta potential significantly decreased in pH of 7.3. Data indicate that at pH 7.3 surface changes of the carcinoma cells are more uniform. This is an advantageous situation to increase the efficiency of drug-cell interactions.

In summary, we demonstrate that Zeta potential measurements of the human lung healthy and carcinoma cells could have a practical value for cancer patients. In this particular case, switching the pH value from 6.2 to 7.3 shows the beneficial condition for drug-cell interaction in chemotherapy. Therefore, the combinatorial approach, the use of alkalizing drugs in combination with various cytotoxic drugs, should be considered in the chemotherapy of cancer patients.

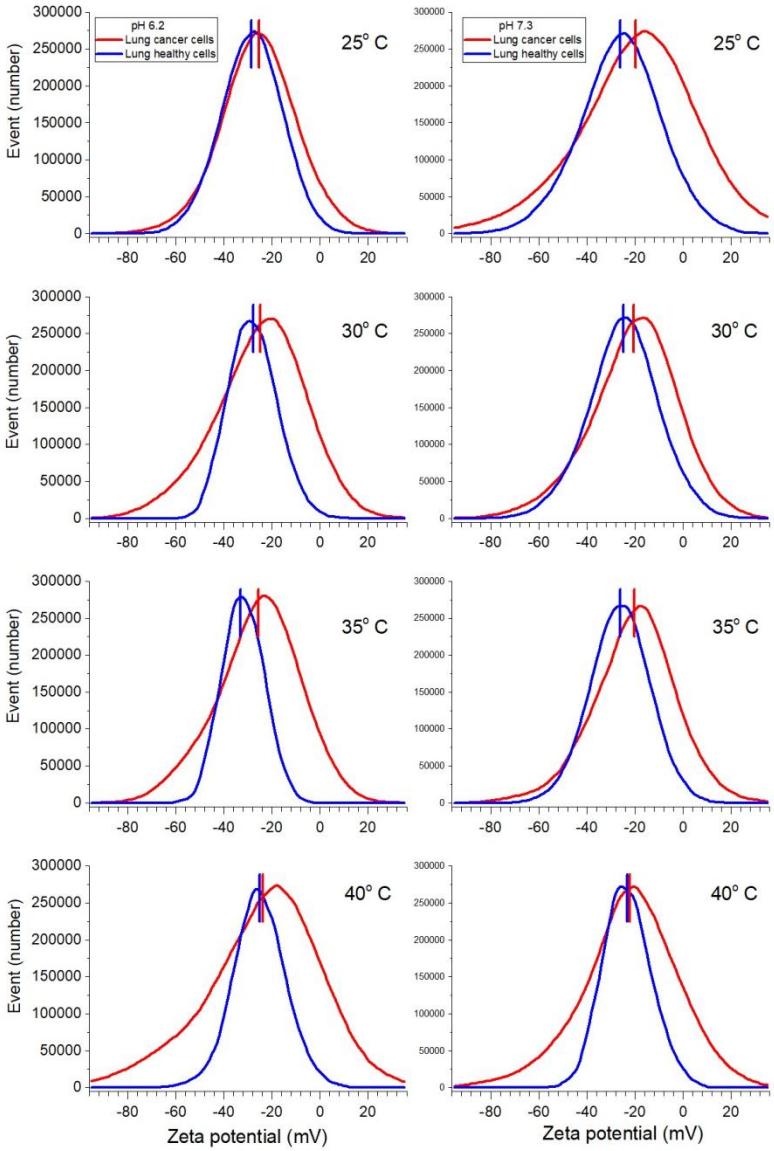

Fig.2. Zeta potential distribution data for human lung healthy and carcinoma cells at different temperatures. Left panel for pH 6.2, Right panel for pH 7.3. Vertical short lines indicate amplitude averaged values for the corresponding Zeta distribution curve.

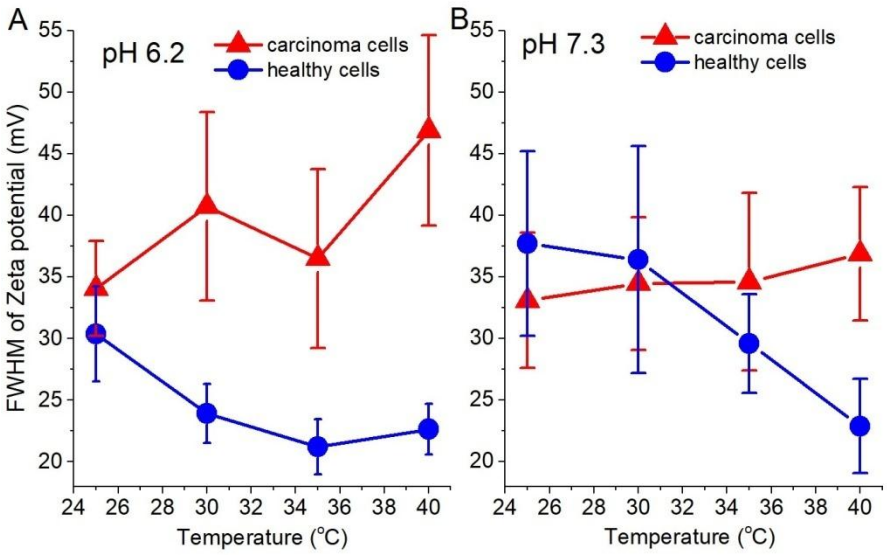

Fig. 3. Heterogeneity of human lung healthy and carcinoma cells assessed from Zeta potentials values. (A) The full width at half maximum (FWHM) from Zeta potential distribution data for human lung healthy and carcinoma cells in pH 6.2 at temperature interval of 25- 45 degree C. (B) FWHM from Zeta potential distribution data for human lung healthy and carcinoma cells in pH 7.3 at temperature interval of 25- 45 degree C. For quantitative assessment of the heterogeneity of the human lung healthy and carcinoma cells, the values of full width at half maximum (FWHM) from Zeta potential distribution data were determined (Figure 3).

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Xülasə

İnsanın ağciyərlərinin sağlam və xərçəng hüceyrələrinin zeta-potensialı: xərçəngin müalicəsi üçün əhəmiyyəti

R.B.Həsənov, L.A.Məlikova, O.K.Qasımov, C.A.Əliyev

İnsanın müxtəlif orqanlarının, o cümlədən ağciyərlərin sağlam və xərçəng hüceyrələri, müxtəlif metabolik aktivliyə malik olur. Sağlam hüceyrələr ilə müqayisədə xərçəng hüceyrələrinin struktuк quruluşu dəyişə bilər. Nətшcədə sağlam və xərçəng hüceyrələri müxtəlif səthi xassələrə malik olur. Bu tədqiqatda insanın ağciyərində xərçəng/karsinoma və sağlam hüceyrənin zeta-potensialı pH 6,2 və 7,3 olduqda, 25°C-45°C temperaturda ölçülmüşdür. pH 7,3 olduqda ağciyərlərin sağlam və xərçəng hüceyrələrin zeta-potensialı təqribən -25 mV (25°C) və -19 mV (25°C) təşkil edir. Göstəricilərdən məlum olmuşdur ki, ağciyərlərin səthi sağlam hüceyrələri karsinomanım səthi hüceyrələri ilə müqayisədə daha neqativ olurlar. Eyni tendensiyalar pH 6,2 oldquda müşahidə edilmişdir, bu hal hipoksik xərçəng hüceyrələrin hüceyrəxarici mühiti üçün xarakterik olmuşdur. Orta turş mühitdə (pH 6,2) ağciyərlərin sağlam və xərçəng hüceyrələrinin zeta-potensialı təqribən -28,5 mV və -25,5 Mv olmuşdur. Zeta-potensialın paylaşdırılması üzrə göstəricilərdən məlum olmuşdur ki, pH 6,2 olduqda, xərçəng hüceyrələrinin səthi eyncinsli olur. Daha az mənfi zeta-potensial sistemin daha az stabil olduğunu göstərir. Əldə edilən nəticələr nano hissəciklərin istifdə edilməsilə dərmanların çatdırılması sistemi aktual məsələdir. Müalicə zamanı nanohissəciklər istifadə edildikdə dərmanların selektiv dərmanların çatdırılması üçün zeta-potensialın düzgün hədlərinə diqqət etmək lazımdır. Nano-hissəciklərin zeta-potensialı hüceyrəyə daxil olmaq və qarşılıqlı təsir üçün zəruri həlledici faktordur. Nano materiallar ilə sağlam və xərçəng hüceyrələrinin qarşılıqlı təsirini zeta-potensial həddinin dəyişməsi üzrə qiymətləndirilə bilər ki, bu da hüceyrələrin dinamik cavabını müşahidə etməyə imkan verir.

Резюме

Зета-потенциалы здоровых и раковых клеток легкого человека: значение для терапии рака

Р.Б. Гасанова, Л.А.Меликова, О.К. Гасымов, Дж.А. Алиев

Здоровые и раковые клетки различных органов человека, включая легкие, имеют различную метаболическую активность. Структурная организация раковых клеток также изменяется по сравнению со здоровыми клетками. В результате здоровые и раковые клетки проявляют разные поверхностные свойства. Здесь значения зета-потенциала рака / карциномы и здоровых клеток легкого человека были измерены при pH 6,2 и 7,3 в интервале температур от 25 ° C до 45 ° C. При pH 7,3 зета-потенциалы здоровых и раковых клеток легких составляют примерно -25 мВ (при 25 ° C) и -19 мВ (при 25 ° C), соответственно. Данные показывают, что поверхность здоровых клеток легких более негативна по сравнению с поверхностью клеток карциномы. Такие же тенденции наблюдались при pH 6,2, характерном для внеклеточной среды гипоксических раковых клеток. В умеренно кислых условиях (pH 6,2) зета-потенциалы здоровых и раковых клеток легких сдвигаются примерно до -28,5 мВ и -25,5 мВ соответственно. Данные распределения дзета-потенциала показывают, что поверхностные заряды раковых клеток более неоднородны при pH 6,2. Менее отрицательный зета-потенциал указывает на меньшую стабильность системы. Полученные результаты актуальны для системы доставки лекарств с использованием наночастиц. В терапии наночастицы должны быть изготовлены с правильными значениями дзета-потенциала для селективной доставки лекарств. Зета-потенциал наночастиц является важным определяющим фактором для входа в клетки и взаимодействий. Взаимодействие здоровых и раковых клеток с наноматериалами можно оценить по изменениям значений дзета-потенциала, которые позволяют отслеживать динамический ответ клеток.

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