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Atomic Force Microscopy in the Study of Cell Membranes Normal Epithelium and Adenocarcinoma Cell of the Large Intestine

Aghajanova AH* and Safarzadeh A

Azerbaijan National Aviation Academy, Baku, Azerbaijan

Abstract

Cancer of the large intestine is approximately 15% of all malignant cancer of the word. In the study of sections of normal epithelial mucosa of the colon revealed that the contours of the cell membrane is precise, well-defined microvilli in size from 5 × 10 to 10 × 10 nm. The adenocarcinoma cell of the large intestine has the morphological changes of the cell membrane. In the cell membrane in contrast to normal epithelial cells of the mucous membrane of the colon appear pores and the degree of change in this indicator may be different.

Keywords: Cancer of the large intestine; Cell membrane; Atomic force microscopy

Introduction

Cancer of the large intestine is approximately 15% of all malignant cancer of the word. Mean age - 60 years. The most common cancer of the large intestine is originated from lower parts of the large intestine (descending colon, sigmoid colon and rectum). In many industrialized countries, cancer of the large intestine is the frequency of one of the highest among all cancers [1].

Statistical date each year ascertain increase in newly diagnosed cases of cancer of the large intestine. Search for a trigger mechanism and risk factors that lead to colorectal cancer are actively learned by many laboratories. Epidemiological data from year to year are added to our knowledge of new factors that contribute to cancer development.

All aspects of early detection, identification, clinical and morphological characteristics, the choice of adequate treatment and its outcome, prognosis factors remain a subject of controversy [2]. For early diagnosis of malignant tumors, which today is essential for their successful treatment must be pass through appropriate screening programs, a surveillance and in-depth systematic surveys of the persons included in the increased risk of malignancy [3]. It should be noted that in the present as a diagnostic test in high-risk of developing cancer can be used by molecular biological research. On the basis of molecular biological techniques can be fairly easy to develop a relatively inexpensive screening, non-invasive research programs based on the identification of modified fragments and combinations of oncogenes. The use of molecular markers of early stages of carcinogenesis could be the basis of new methods of screening, signs of malignant growth to the formation of its morphological manifestations.

Cancer of the large intestine develops in accordance with the fundamental laws of growth and spread of malignant tumors, the relative autonomy and unregulated growth of the tumor, the loss of organ and histogenic structure, reducing the differentiation of tissues. At the same time have its own features. Thus, the growth and spread of colon cancer are relatively slower than, for example, stomach cancer. Over a long period of tumor is within the body, not extending to a depth of bowel wall more than 2-3 cm from the visible border. The slow growth of the tumor is often accompanied by local inflammatory process, passing on the adjacent organs and tissues. Within the inflammatory infiltrate in the adjacent organs are constantly sprouting cancer centers, which contribute locally advanced tumors without distant metastases. Among the malignant epithelial tumors of the most common is adenocarcinoma - it accounts for more than 80% of all cancers of the large intestine. In predictive knowledge to the degree of differentiation (high-, medium-and high-grade adenocarcinoma), depth of germination, clarity of boundaries of the tumor, the frequency of lymphatic metastasis is very important. In patients with well-differentiated carcinomas more favorable prognosis than patients with poorly differentiated cancer.

The mechanism of occurrence and development of many pathological conditions, including malignant growth is associated with disruption of the structure and properties of biological membranes [4,5].

Atomic force microscopy, invented in 1986, is widely used for imaging materials at the nanoscale. Its resolution is similar resolution electron microscopy, but, in contrast, does not require a vacuum and can therefore be used for the study of living specimens. However, conventional AFM requires several minutes to get a single image, and therefore it cannot record a sequence of rapidly occurring events [6]. The AFM have optimized for the study of living cells. Atomic force microscopy uses a cantilever equipped with a probe, which "feels" the surface of the sample. The forces between the tip and the sample can be measured as the probe moves over the sample, thereby revealing the surface topography [7,8].

Methods of atomic force microscopy are increasingly being used in biology, medicine and pharmacology. Atomic force microscope (AFM) has several advantages over optical and scanning electron. First, it allows obtaining a true three-dimensional relief of the surface. Secondly, when you use it is not required to sample conducts electricity. In addition, measurements can be performed not only in vacuum but also in the air in the atmosphere of any gas or even a drop of liquid. The

***Corresponding author:** Aghajanova AH, Azerbaijan National Aviation Academy, Baku 1045, Azerbaijan, Tel: +994 12 497 28 29; E-mail: aghajanovaarifa@gmail.com

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last circumstance opens up wide possibilities for the study of organic molecules and living cells [9,10].

Obviously, on the basis of the AFM is possible to develop a methodology to assess the clinical morphofunctional activity of tumor cells, based on the morphometric of the large number of simultaneously visualized cells. Investigation the structural features of the membrane of tumor cells, as well as quantitative estimation of properties of the cell membranes is very important. The study is promising, since a change in structural of the cell is the result or may result in development of certain diseases, including cancer.

The purpose of the study, to reveal the nature of structural defects in the cell membranes adenocarcinoma cells of colon, establishes differential diagnostic criteria using atomic force microscopy.

Materials and Methods

Been investigated or postoperative and endoscopic biopsy material of 15 patients with adenocarcinoma of colon and 5 patients with non-tumor diseases. Further, the material obtained from tissue samples, the intestinal epithelium and tumors were prepared in cytological smears on coverslips. Smears were dried in air and were examined by atomic force microscope Asylum MFP-3D™ Stand Alone AFM.

Results and Discussion

All the materials investigated in parallel by routine histology and confirmed the diagnosis. The study by atomic force microscopy was carried out starting with a smaller increase then selected sites for detailed study in a large increase.

Mucosal epithelium of the colon - a single-layer prismatic. It is composed of columnar epithelial cells, goblet cell, and individual endocrine cell and poorly differentiated (cambial) cells. The large number of goblet cells is in correlation with the need to allocate mucous secretion, which facilitates the mass of food through the intestines. In his own record of the mucous membrane is highly developed defense mechanism against microbes. There are numerous lymphocyte accumulations. For research in atomic force microscopy were selected epithelial cells.

In the study of normal colon epithelial cells in 2D and 3D, with an increase in 5micron on the surface of the cell membrane are identified protrusions and depressions (Figure 1).

Then there was the chosen site of the normal epithelial cells of the intestinal mucosa, respectively, in the dimension 2D and 3D, with an increase in 2micron. In the study revealed that the surface of the cell membrane is rough, with protrusions and depressions of various sizes. Protrusions were painted in bright colors, parts of depressions painted in dark colors (Figure 2).

In the learning cell membrane normal epithelial cell of the colon in increase 450 nm we can see that on the surface of protrusion, depressions clearly visible microvilli (Figure 3).

Next, to clarify the dimensions of the microvilli watched section of the same sample. In the study of sections of normal epithelial mucosa of the colon revealed that the contours of the cell membrane is precise, well-defined microvilli in size from 5 × 10 to 10 × 10 nm (Figure 4).

AFM study of the relief of the cell membrane of colon adenocarcinoma revealed significant differences adenocarcinoma cells and normal cells of the colon. In the cells of the colon adenocarcinoma cell surface was rough, with protrusions and depressions. The size of protrusion and depressions 2 micron. The number of microvilli on the surface membrane of the cell adenocarcinoma compared with

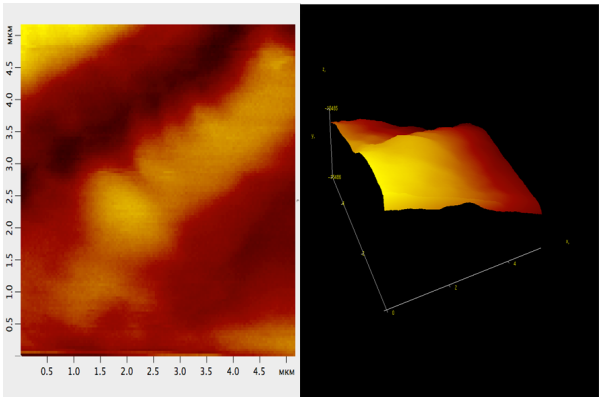

Figure 1: Area of the normal epithelial cells of the mucous membrane of the colon, respectively, in 2D and 3D, with an increase in 5 micron. The surface of the cell membrane with protrusions and depressions.

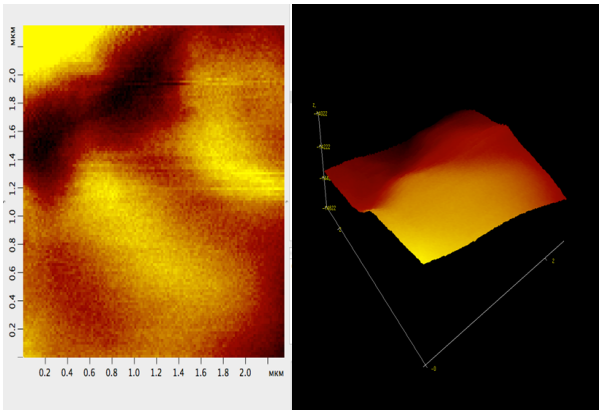

Figure 2: The area of the normal epithelium of the intestinal mucosa, respectively, in 2D and 3D, with an increase in 2 micron. The surface of the cell membrane is rough, with protrusions and depressions. Light-colored areas is protrusions, dark-colored areas, depressions.

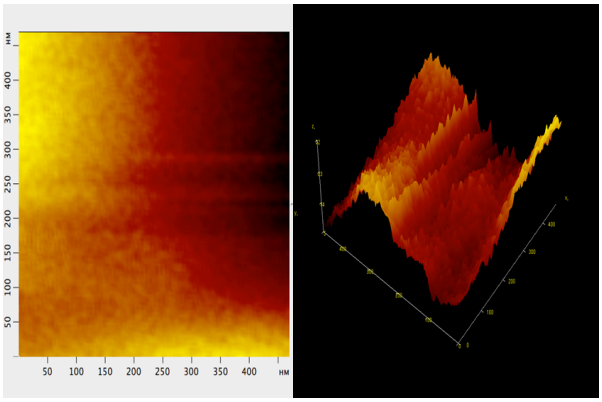

Figure 3: The area of the normal epithelial cells of the mucous membrane of the colon respectively in 2D and 3D, with an increase of 450 nm. The surface of the cell membrane protrusion, depressions and on the surface of protrusion, depressions clearly visible microvilli.

normal epithelial mucosa of the colon is less, the size decreased. In the depressions determine the pore (Figure 5).

In section of a cell adenocarcinoma of the colon in a 2D format, with an increase in 4.5 micron seen visible pores on the surface of the cell adenocarcinoma. The size of the pore on the surface of adenocarcinoma cell of colon is 26 nm × 0.6 micron (Figures 6 and 7).

The data presented suggest that adenocarcinoma of the colon have the morphological changes of the cell membrane of cells. In the cell membrane in contrast to normal epithelial cells of the mucous membrane of the colon appear pores and the degree of change in this indicator may be different.

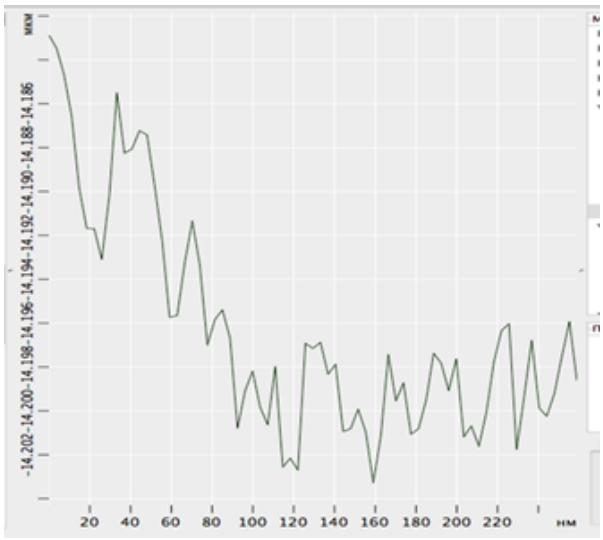

Figure 4: Section of the normal epithelial cells of the mucous membrane of the colon. Precise contours of the cell membrane are well defined, visible microvilli measuring from 5x10 to 10x10 nm.

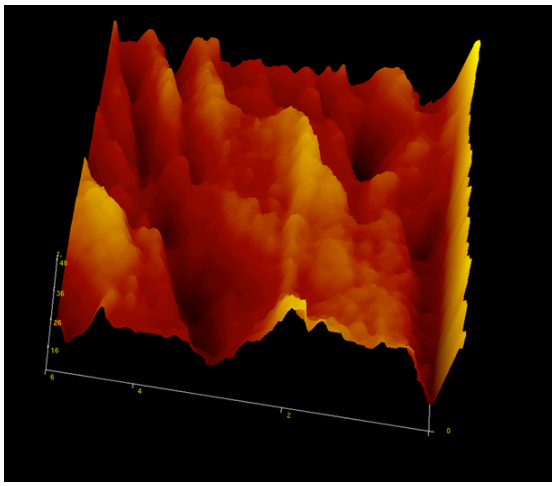

Figure 5: Section of a cell adenocarcinoma of the colon in 3D, with an increase of 6 micron. The surface of the cell membrane is rough, with protrusions and depressions. Light-colored areas are protrusions, dark-colored areas is depressions. The size of protrusion and depressions 2 micron. The number of microvilli on the surface membrane of the cell adenocarcinoma compared with normal epithelial mucosa of the colon is less, the size decreased. In the depressions defined pores.

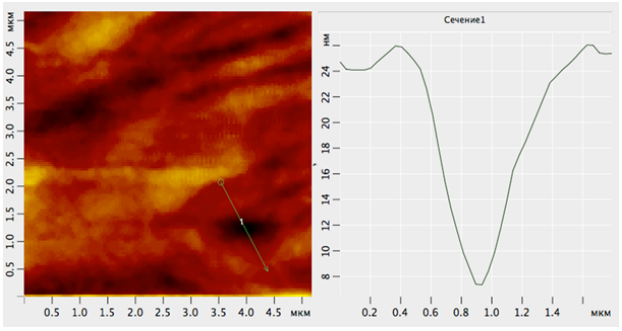

Figure 6: Section of a cell adenocarcinoma of the colon in a 2D format, with an increase in 4.5 micron. On the surface of the cell adenocarcinoma of visible pores. Section on cell adenocarcinoma of colon seeing pore size 26 nm × 0.6 microns.

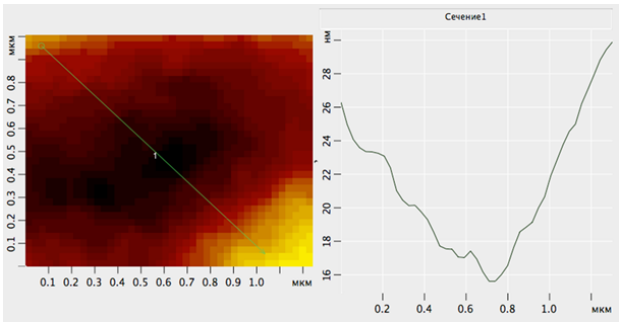

Figure 7: Section of a cell adenocarcinoma of the colon in a 2D format, with an increase in 1 micron. On the surface of the cell adenocarcinoma of visible pores. Section on cell adenocarcinoma of colon is seeing pore size 26 nm × 1 micron.

The perhaps surface change of the cell membrane is component of other change in the structure of the cytoskeleton and one of the factors explaining their infiltration into neighboring tissues and organs, the formation of metastases. The study of structural and morphological properties of membranes allows the future to distinguish cancer cells from healthy controls. And that could lead to the development of new methods of prevention and diagnosis of cancer. Thus, atomic force microscopy - not just a method of visualization of the objects with high resolution, but also a very promising way to study local morphological and biophysical properties of individual cancer cells. However, not enough research on issues of differential diagnosis of cancer of various sites, at the present time, atomic force microscopy is still very far from the state of routine methods. AFM is developing new methods for detecting, diagnosing and treating cancer.

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