Characterization of Gastrointestinal Cancer Cells Invasiveness by Estimation of Their Motility

R. Petrolis[1, 4,*], R. Ramonaitė[2], D. Jocevičius[2], G. Kiudelis[3], L. Kupčinskas[2], A. Kriščiukaitis[1,4]

[1]*Neuroscience Institute of Lithuanian University of Health Sciences, Lithuania*
[2]*Institute for Digestive Research of Lithuanian University of Health Sciences, Lithuania*
[3]*Department of Gastroenterology of Lithuanian University of Health Sciences, Lithuania*
[4]*Department of Physics, Mathematics and Biophysics of Lithuanian University of Health Sciences, Lithuania*
[*]*E-mail: robertas.petrolis@lsmuni.lt*

Introduction. Tumour cell migration to the healthy tissue leads to the emergence of metastases. Cancer cells migrate in various ways, according to cell type and degree of differentiation. At least two distinct types of cell migration (mesenchymal at speed < 1 μm/min and amoeboid at speed ~ 10 μm/min) are known [1]. Chemotactic mechanisms are important in cell motility. Chemotactic and platelet-derived growth factor signalling is influencing cancer cell invasiveness what results in metastasis formation [2]. In recent years collective cell migration – the ability of groups of cells to move together and simultaneously affect the behaviour of one another – is recognized as distinct type cell migration [3]. Cancer cells possess a unique ability to adapt to different environmental conditions, assuming different morphologies and migration characteristics in order to stay motile [4]. So quantitative estimates of cell motility could be important features reflecting tumour cell invasiveness and process of metastasis formation.

The aim of this study was to develop a method for quantitative evaluation of cultured cell migration, based on long-term continuous non-stained gastrointestinal cancer cells imaging.

Methods. Six human gastrointestinal cancer cell lines HCT116, MKN28, RKO, Caco2, AGS and SW620 were maintained in F-12 medium supplemented with 10% fetal bovine serum, and 1% antibiotics (100 U/ml penicillin, and 100 μg/ml streptomycin) in a humidified air containing 5% CO2 at 37°C. The cells were trypsinized and 50 μl of the cell suspension was added to 75 cm^2 flasks with medium for the migration testing.

Continuous long-term cell culture imaging was performed several times for each flask. Actual number of tests depended on viability of cells and was ranging from 1 to 5. Phase-contrast images of cells were taken using OLYMPUS IX71 light microscope equipped with Q IMAGING EXI aqua camera at 1392x1040 pixels resolution. We used x10 magnification at which each pixel was representing 0.6 μm x 0.6 μm real area. Images were taken every 30 seconds during a period of 3-4 hours and stored as series. Such frame

rate is assuring enough resolution to observe fast amoeboid cell migration and at the same time saves computation and storage resources.

Subtracting two neighbouring images in series revealed cell movement events. Image histogram adjustment of all images was done during preprocessing. Difference images were converted to binary format using threshold determined by Otsu's method for detecting significant difference in pixels showing moved cell. Mathematical morphology operation "image opening" was used to filter out small particles. Areas containing at least 300 white pixels (about 1/5 of single cell area) were considered as cell movement event. Sum of all pixels in binary images was calculated for every difference image and was used as function to recognize cell movement events. All cells in our experiments were of about the same size (covered area of between 1000 and 2000 pixels) and all movement events were similar in values of sum of all pixels. Concurrent two or three cell movement events were simply increasing value of sum of all pixels by factor 2, or 3, depending how many events were concurrent.

Approximate cell count was determined as ratio of area covered by cells and average area covered by one cell. Areas were automatically determined after image contrast enhancement and mathematical morphology operations "image closing" and "filling the holes". Example of detection of area covered by cells is given in Fig.1.

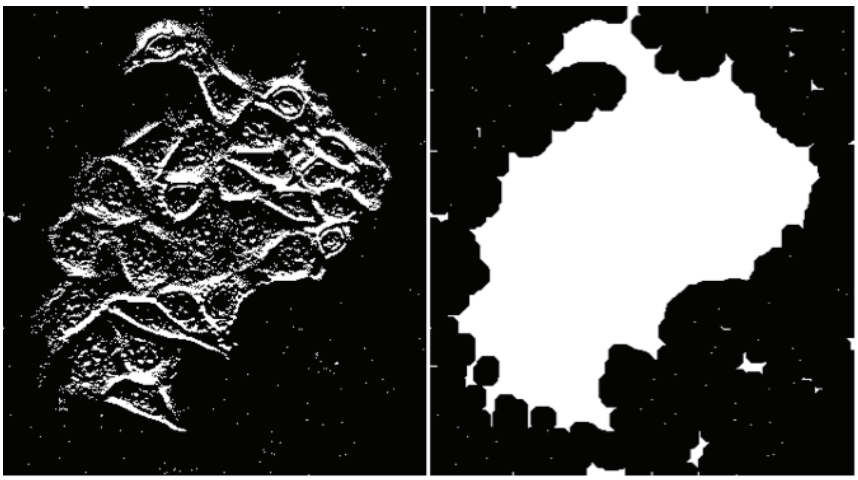

Fig. 1. Example of detection of area covered by cells: enhanced contrast image on the left; binary image after "image closing" and "filling the holes" operations on the right

Results. Typical images of cultured cells MKN28 are presented in upper part of fig.2. The pictures are superimposed with binary images indicating cell movement events. Sum of the binary image pixel values, calculated from 30 ordinary images from the series is presented in the bottom graph. Two peaks,

indicated by the arrows correspond to the cell movement events reflected in binary images. Observed cell movement events in our experiments one can describe as reshaping and shift of single cell in a distance of about the size of single cell. We did not observe long distance cell movements, which would exceed the single cell size. Also we did not observe any group cell movements.

As result we proposed a quantitative estimate – average number of cell movement events per hour in population of 1000 cells. Values of this estimate for all investigated cell lines are presented in Table 1.

To see the distribution of cell movement events over the whole observed area we merged all binary images containing indicated cell movement events into one and superimposed with initial phase contrast cell culture image. The example of such combined image is presented in Fig.3. Such representation revealed that cell movement events take place only in sparse distributed cell areas.

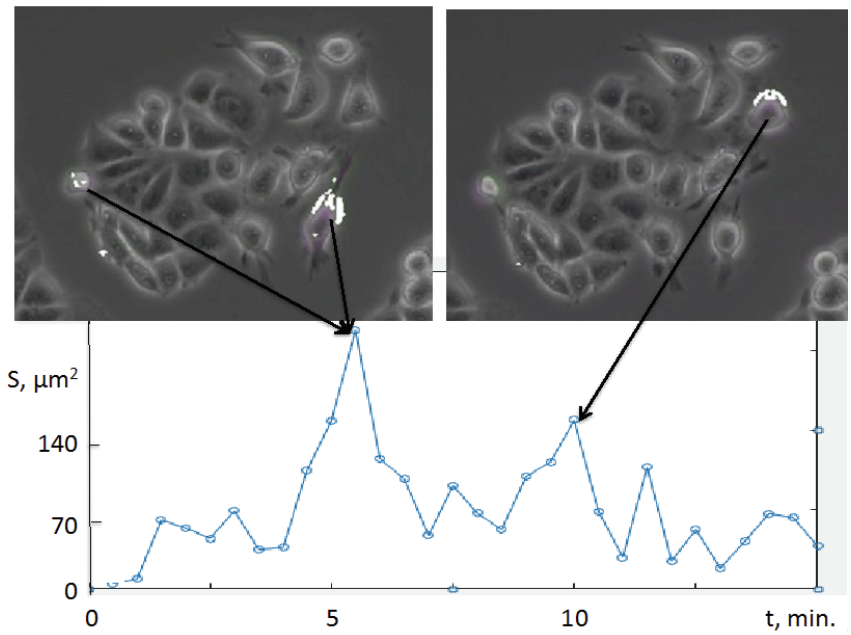

Fig. 2. Typical images of cultured cells MKN28 superimposed with binary images indicating cell movement events (upper images). Sum of pixel values in binary images at the moments of cell movement events (bottom graph)

Discussion. In this study, we found that the values of average cell movement events were different in gastrointestinal cells lines, i.e. gastric cancer cell lines had more movement events compared with colon cancer cell lines. Similar findings were observed in the other studies where authors shown that tumor cells move the different ways and speed [1,5-9].

The limitation of our study firstly lies in the technical conditions. We did not have continuous controlled incubating conditions during the imaging. That limits the time till solution with cells gets acidified. However division of the cells, what confirms their well being, was observer even during the last hours of imaging.

Table 1. Motility of the cells in gastrointestinal cancer cells lines

Cell Line		**Number of tests**	**Number of cell movement events per hour in population of 1000 cells (mean (SE))**
Gastric cell lines	MKN28	5	91,2 (32,9)
	AGS	5	70,0 (23,9)
Colon cell lines	HCT116	5	56,2 (15,8)
	RKO	4	15,89 (9,5)
	SW620	1	13,17 (--)
	Caco2	3	2,99 (1,1)

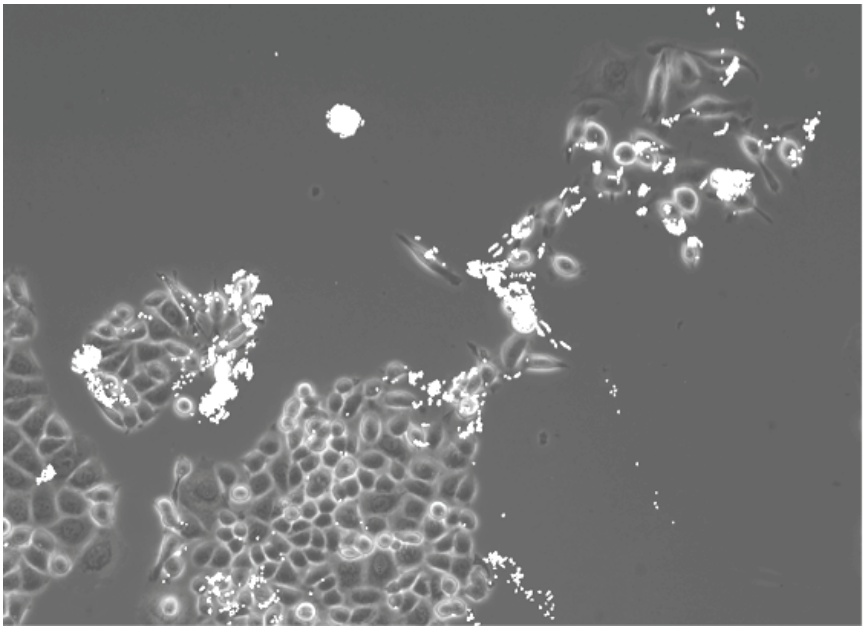

Fig. 3. The example of combined cell culture and cell movement events image revealing their distribution over the whole observed area.

Conclusions, future work. Automatic determination of cell motility estimates in cultured cells opens new possibilities in investigations of viability of the cells. In case of cancer cells it gives quantitative estimates of invasiveness and opens new possibilities for development of treatment means

and methods. Cell movement in many cases is stimulated by some chemotactic processes. So, we intend to apply chemotactic test to our future investigations.

Acknowledgments. This study was supported by the Lithuanian state Science and studies foundation (Title of project: "Functional role of miR20b, miR451, miR29c, miR125b in pathogenesis of gastric and colorectal cancer", Project Nr. MIP-007/2014).

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[3]Department of Gastroenterology of Lithuanian University of Health Sciences, Lithuania
[4]Department of Physics, Mathematics and Biophysics of Lithuanian University of Health Sciences, Lithuania

A method for automatic determination of quantitative estimate of cultured gastrointestinal cancer cells motility is proposed to estimate their invasiveness. The method is based on mathematical morphology methods applied for cultured cell image series taken during long term (about 3 - 4 hours) continuous imaging.