Rapid In Vitro Screening Test of Tumour Cells Migratory Reactions to Potential Migrastatics

Markéta Šuráňová [1,2], Jan Brábek [3], Irena Štenglová Netíková [4], Radim Chmelík [1,2] and Pavel Veselý [2]

1 Institute of Physical Engineering (IPE), Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic
2 CEITEC - Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic
3 Department of Cell Biology, and Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec (BIOCEV), Laboratory of Cancer Cell Invasion, Charles University, Prague, Czech Republic
4 General University Hospital in Prague, Department of Clinical Pharmacology and Pharmacy, Prague, Czech Republic

ABSTRACT

Solid tumor metastases particularly the late ones cause the majority of cancer-related deaths. Prevention of their occurrence has been lacking suitable medication. The novel conception of migrastatics stems from the knowledge of the link between cancer cell metastatic potential in vivo and their enhanced migratory activity in vitro and looks for a workable solution based on inhibition of cancer cell migration as probed in vitro. Repurposing medicaments for exploitation of their side effects for migrastatic/migration inhibiting activity appeared to be the easiest way to the fast progress in the fight against metastases. This situation led us to think about a design of a suitable first sieve for catching the right migrastatic candidate. For registration of live cell activities, we used Q-Phase by Telight, Brno, the Czech Republic, which is a Coherence-Controlled Holographic Microscope with Holographic Incoherent Quantitative Phase Imaging. It provides a non-invasive method of monitoring cellular events, especially migration and growth with changes in cell morphology during a 20-hour, approximately the length of the cell cycle, time-lapse follow-up. This microscopical biotechnology enables the most reliable and accurate automatic cell segmentation and monitors growth, morphology, and positional changes over time. Then the automated image analysis of the whole cell population for speed of migration of the individual cells and evaluation of their migratory and growth behavior is crucial for the assessment of the overall impact of the examined putative migrastatics. On the other side, it is complemented by watching for a possible rare occurrence of an invasive cancer cell phenotype induced by the stress elicited incidentally by the tested medicament.

METHODS AND MATHERIAL

Q-PHASE (Telight, Brno, Czech Republic), a commercially available **CCHM**, was employed for the **Holographic Incoherent Quantitative Phase Imaging (hiQPI)** of cells. The CCHM is based on an off-axis setting with an incoherent light source. The incoherent CCHM source enables high-quality **hiQPI** without specles and parasitic interferences. Thanks to the off-axis setting, only one hologram is required for image reconstruction and so fast processes can be observed.

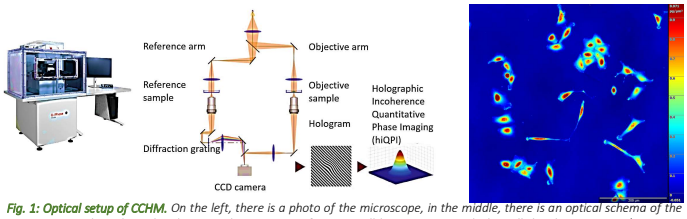


Fig. 1: Optical setup of CCHM. On the left, there is a photo of the microscope, in the middle, there is an optical schema of the microscope and on the right, there is a hiQPI image of cancer cell lines HT1080 with dry cell distribution in pg / µm2. An objective lens is 10x0.3

Live Human tumor cells in vitro A549 (amoeboid movement) and HT1080 (mesenchymal movement) were examined by CCHM for 20 hours at 5-minute intervals. For these cell lines, Ibidi µ-Slide VI0.4 was used for 20 hours by CCHM. Belumosudil (BEL), Doxycycline (Doxy), and Midostaurin (Mid) were selected to be tested as possible migrastatics for this research. It is a combination of these drugs to compare the effect of the drugs on the invasiveness of amoeboid and mesenchymal cells. Combinations of migrastatics are chosen as follows: doxycycline (inhibitor mes. inv.)+belumosudil (inhibitors am. inv.) and midostaurin (inh.mes. inv.)+belumosudill (inhibitors am. inv.).

RESULTS

The dynamics of migratory behavior of selected cell lines after medicaments application were evaluated. The graphs below show measurements of the dynamic properties of cells HT1080 after the application of **migrastatics.** The following data were measured using a CCHM Q-PHASE and an objective lens 10x0.3.

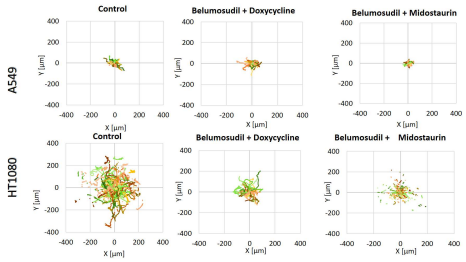


Fig. 2: Motility rose graphs of cell lines HT1080 with migrastatics. Cell trajectory graphs from hiQPI records. The individual tracks of the 20-hour scan were shifted to a common origin [0,0]. Axis represent distances in µm. An objective lens 10x0.3.

The greatest reduction in motility was caused by the application of the combination of two migrastatics - belumosudil and midostaurin in A549 cells, as well as in HT1080 cells.

For CANCER CELL INVASIVE PHENOTYPE (CCIP) evaluation, parameters such as Euclidean distance, Meader index, circularity and perimeter are evaluated. The concept of a rapid indicative 2D in vitro test for the suitability of a drug, selected as a potential migrastatics, for entry into further preclinical testing is based on CCHM biotechnology. This enables the migration and changes in cell morphology to be monitored as well as their weight checked during the 20-hour test (approximately the length of the cell cycle). Automation of the evaluation of the obtained data will reveal statistically significant changes in cell migration and growth, as well as the occurrence of an invasive phenotype of tumor cells as a result of the stress induced by the test substance.

First, the hiQPI records are shown in chronological order. Furthermore, individual cells are automatically segmented by color. They are colored according to the ratio of Euclidean distance and the Meandering index.

Color segmentation of the cell	Euclidean distance	Meandering index
green	high	high
red	low	high
blue	low	low
violet	high	low

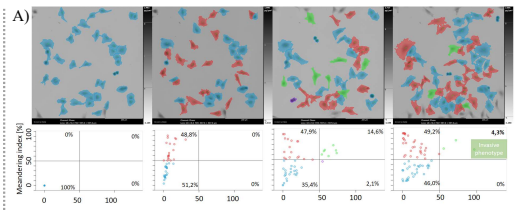
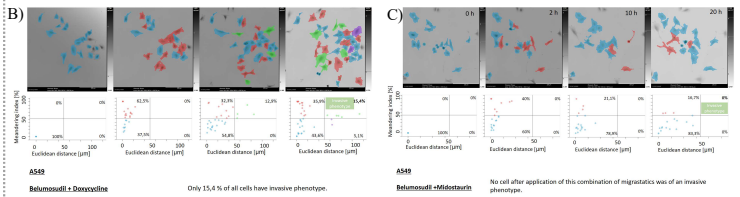


Fig. 3: Evaluation of phenotypes in A549 cells. A) There is hiQPI and graphs of control. B) Time plots of A549 cells after belumosudil and doxycycline application are shown here. C) Time plots of A549 cells after application of belumosudil and midostaurin are shown here. hiQPI images are taken with a 10x0.3 lens. The graphs shown below are from SophiQ software..

Only 4.3% of the cells had an invasive phenotype, but after the application of belumosudil and doxycycline there was an increase to 15,4% in the invasive phenotype, on the contrary, after the application of the combination of drugs belumosudil and midostaurin there was a complete reduction of invasiveness.



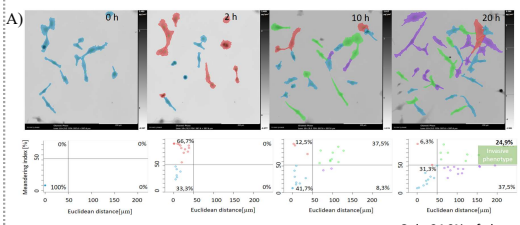
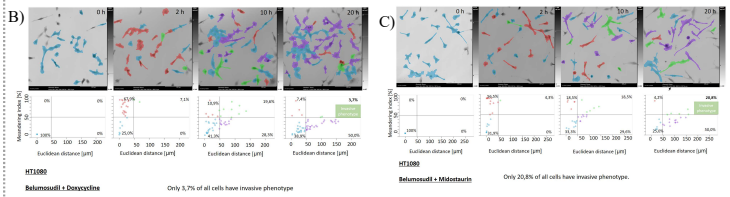
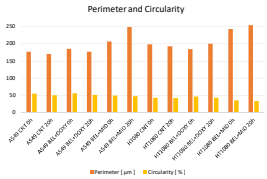


Fig. 4: Evaluation of phenotypes in HT1080 cells. A) There is hiQPI and graphs of control. B) Time plots of HT1080 cells after belumosudil and doxycycline application are shown here. C) Time plots of HT1080 cells after application of belumosudil and midostaurin are shown here. hiQPI images are taken with a 10x0.3 lens. The graphs shown below are from SophiQ software..

Only 24.9% of the cells had an invasive phenotype in the control, but after the application of belumosudil and doxycycline there was a decrease to 3.7% in the invasive phenotype, on the contrary, after the application of the combination of belumosudil and midostaurin there was only a slight decrease in invasiveness to 20.4%.





Tab. 1: Graph of Perimeter and Circularity. The data are from the beginning and end of the measurement. The data are from hiQPI records. The individual tracks of the 20-hour scan were shifted to a common origin [0,0]. Axis represent distances in µm. An objective lens 10x0.3.

This graph shows that BEL+MID had the greatest effect on HT1080 cells, which caused more rounding of the cells. After this combination, however, the A549 cells had a larger perimeter and were less rounded.

The combination of belumosudil and midostaurin had the best effect on the invasiveness and motility of A549 cells. On the contrary, the combination of belumosudil and doxycycline had the best effect on HT1080 cells.

This methodology can be used to evaluate the invasiveness and movement of other living tumor cells and can also be used for other potential migrastatics, which with their secondary effects could help the invasion of tumor cells from the primary tumor.

CONCLUSIONS AND ACKNOWLEDGMENTS

We successfully employed the methodology of CCHM hiQPI for evaluating the impact of candidate migrastatics on cancer cell dynamics of migration as well as morphological plasticity to reveal substantial traits of cancer cell invasive phenotype. These findings complemented with an option of weighting cells for controlling growth or invasive phenoptype by this method a promising approach to a fast early appreciation of potential migrastatics.

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Mgr. Markéta Šuráňová | Experimental Biophotonics research team, Faculty of Mechanical Engineering, Brno University of Technology (BUT), Czech Republic | Marketa.Suranova@vutbr.cz