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ESPI-Analysis of induced stress on cellular systems

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Abstract:
During the last years, various approaches on an individualized drug therapy for benign cells have been researched. However, due to the complex topic a universal approach has not been found until this point. Commonly, the effect of cytotoxic drugs on benign cells is mostly the same as to normal cells and the actual effect of cytotoxic agents on patient cells still can't be predicted. In order to reduce unwanted side effects or unspecific drug reactions a test system for patients which allows to analyses the interaction between cytotoxic agents and the cells of the patient is need. Furthermore, this should also include an adequate measurement system which is capable to work in a natural environment and without any additional preparation. In terms of this work, a first proof of concept with cultured benign cells and different cytotoxic agents is presented while monitoring the obtained displacement using a modified electronic speckle pattern interferometry (ESPI).

Key words: ESPI, Interferometry, Cytotoxic Agents, Cellular Displacement

Introduction

In order to research cell-drug-interaction after the incubation with cytotoxic agents and the resulting cellular behavior of degenerate as well as normal cells in-vivo-systems (e.g. model organism mouse or makak) are commonly used. One of the main advantages is that the cell-cell-interaction is preserved and therefore allows to estimate the toxicity as well as the potency of the substance. Furthermore, these tests also allow the metabolism of so-called "prodrugs", with the exception that numerous metabolic pathways of humans and model organisms differ dramatically, as the TGN1412 study from the year 2012 proved. The in vivo experiments yielded promising results so that the substance was approved for the Phase I study. In this way, the drug released serious side effects within a few minutes. The reason for this was a difference in the amino acid sequence of the CD28 molecule. Especially since this study it was shown that an adaptation of the cytostatic effect of in vivo experiments can have heavy consequences for the patients [1].

In order to examine the actual impact of a cytotoxic agent, a test system is required which allows an investigation in a human organism. In initial experiments cultured transformed cells were provided with various cytotoxic agents and their interaction were observed by ESPI. For this, a test setup was developed, which allows an adaptation of optical measurement and the study of cells in an almost natural environment as well as the detection of surface changes in the micrometer range. First tests were used for the generation of reference models in terms of induced surface modification of the cells. Following, to analyze the behavior of the cell body the influence of different chemotherapeutic agents under different concentrations were obtained. The first series of experiments initially yielded very promising results. For the used reagents it was already possible to measure individual stages of the cellular reaction by using the ESPI technique.

Methods

For the analyzation of the cellular response after addition of a cytotoxic agent HeLa cells and L929 cells were used as a model system. The chemotherapeutic agents Halaven® was used as a cytotoxic agent. Halaven® contains Eribulin as active ingredient and is usually applied for the treatment of advanced metastatic breast cancer [2]. For the observation of the cell reactions, adhesion media made of silicon were used. The adhesion media was placed in a reaction chamber and filled with culture medium without FBS. The respective chemotherapeutic agent was applied

into the chamber. The reaction states in form of object images are recorded in an interval of 3 to 5 min. The added volumes of chemical agents depends on the corresponding documents of the drug [2].

Results and discussion

In the performed EMBRACE-study of 2011, the efficacy of Eribulin was conducted with 762 patient worldwide. Result of this study was that the one-year-survival rose to 53.9 % after the application of Eribulin [5]. Eribulin binds to the beta subunits of the tubulins and overlaps the GTP binding site at the same time. Thus, the tubulin polymerization is inhibited and the tubulins are degraded into non-productive aggregates. Thereby, the spindle apparatus can't be formed and the inhibition of mitosis takes place. Therefore, apoptosis is induced by cellular mechanisms [2]. As already mentioned, Halaven is used to treat metastatic breast cancer. The female breast consists of adipose tissue, connective tissue and glandular tissue. In principle, these malignant degenerations belong to epithelial tumors. In order to analyze the effectiveness and selectivity of Eribulin a degenerated epithelial cell line (HeLa-cells) and a connective tissue cell line (L929 cells) were selected. The obtained experimental results for a single HeLa cell after 30 min incubation with Halaven® are shown in figure 1.

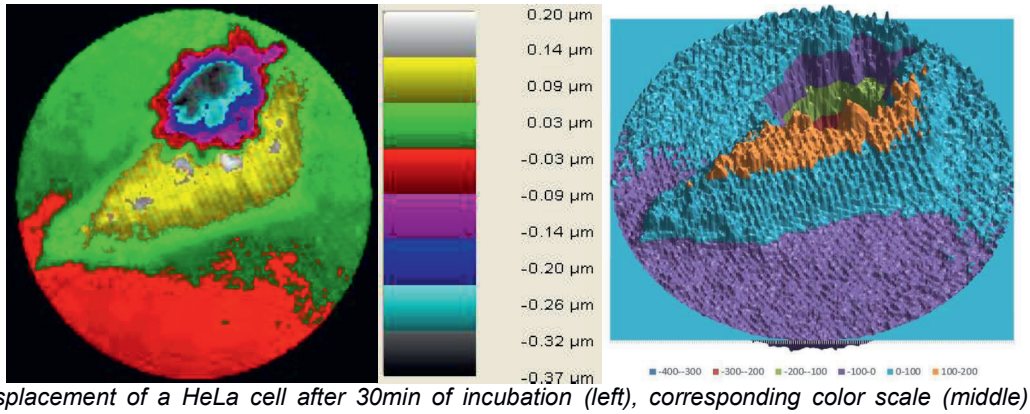

Fig.1.Displacement of a HeLa cell after 30min of incubation (left), corresponding color scale (middle) and 3D image (right)

The outlines of the cells are clearly visible and also exhibit scant background noise. Hereby, the ESPI detected for the HeLa cells large positive (red and green) as well as circular negative (violet and blue) deformation of the cellular body. This is caused by the apoptotic reactions and the resulting detachment of the cell itself. Nevertheless, the overall displacement of the HeLa cell could be estimated with approximately 320 nm in total. In this case, Eribulin has successfully interact with the degenerated epithelial cell. In order to analyze the selectivity of the cytotoxic agent, the interaction of Eribulin with cells of the connective tissue was researched. These cells are also a main component of the female breast. For this, L929 cells were used. For a reproducibility of the performed experiments the same parameters as before were used. In Figure 2 the displacement of a single L929 cell after 30 minutes of incubation with Halaven® as a result of the ESPI measurement is shown.

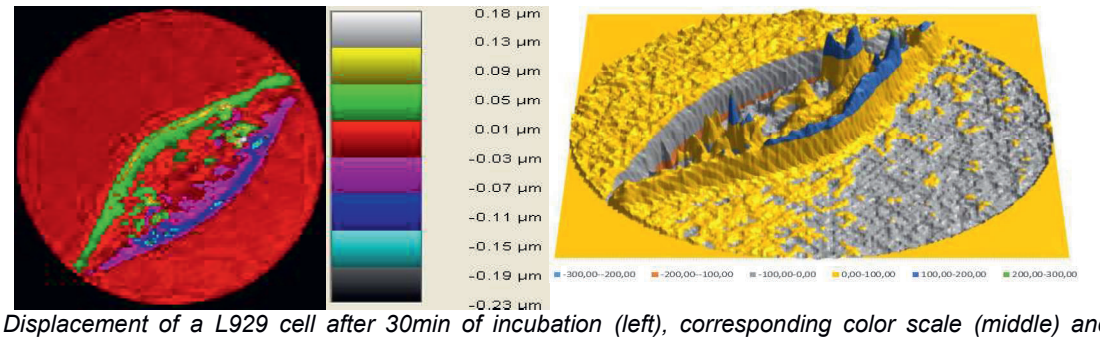

Fig.2.Displacement of a L929 cell after 30min of incubation (left), corresponding color scale (middle) and 3D image (right)

After a reaction time of 30 min the outline of the cell are clearly visible and only minimal negatives displacements (blue colored) were measured, which are recirculate to the minimum flows in the measurement chamber. Compared to the measurement of the single HeLa cell the measured negative displacements are significant smaller. From these results it can be concluded, that Eribulin does not interfere with the L929 cell.

Conclusion

Since the removal of human epithelial cells from a cervical cancer more than 60 years have passed. With the help of these cells, the first cell line was established and has been used worldwide by scientists to investigate cell behavior in various fields of science [3], [4]. Furthermore, no reliable therapies or explanations could be developed, which explain why, for example, chemotherapy is only partially effective. Within this work, a new strategy for the investigation of cell behavior after application of a cytotoxic agent was demonstrated. After injection of the drug Halaven®, an apoptotic reaction of the HeLa cell could be obtained as expected and no reaction of the L929 cell was obtained. For further studies of the cell-drug-interaction and the cellular behavior of degenerate and normal cells, evaluations of whole organoids as well as long-term experiments by using ESPI are required. The main advantages of this method are the abilities to observe the biological samples in real-time, without sample contact and without sample destruction. Furthermore, the micro-ESPI allows an analysis of human cells directly in the medium without affecting the result. This allows to examine the cells in an almost natural environment which provides the fundamental principle to analyze sample of cancer patients in the future.

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