Companion Diagnostics for Breast Cancer Chemotherapeutics

Monica Tawadros, BS[1] , Michael Morin, PhD[1] , Peter Gaines, PhD[1] , Abiche Dewilde, PhD2 [1]
1 Department of Biological Sciences, University of Massachusetts Lowell;
2 InVitroMetrix Corporation, Lowell MA

Technology

We have developed a multi-well cell-based sensor that can monitor real-time biological changes in living cells, such as mass redistribution and viscoelasticity. This system provides unique kinetic information regarding the phenotypic change in the cells post treatment (Fig 1).

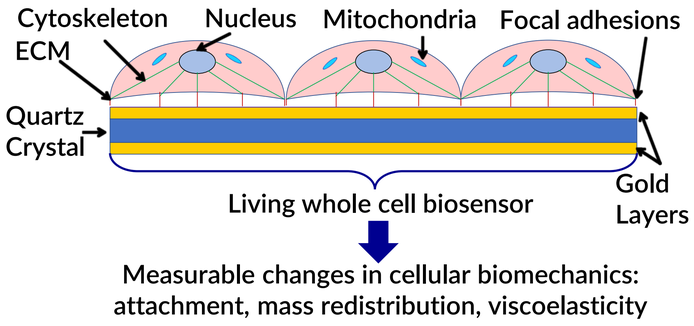

Figure 1. Quartz Crystal Microbalance method of detection

Our biosensor allows the investigation of downstream signaling in whole living cells maintained in culture conditions. The experiments are conducted with the same technique as a cell culture assay that take place in a cell culture well plate (Fig 2). This environment and method allows the cells to respond to the drug that is added. Integrated in each well is a piezoelectric sensor that the cells adhere to (Fig 1). This enables us to measure in real time the changes that occur within a cell in response to drugs or agent (Fig 3, 4, 5).

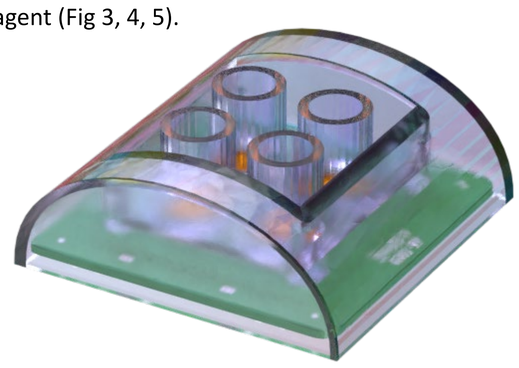

Figure 2. Discovery-Q Device, in incubator, with humidity, temperature and CO_2 control

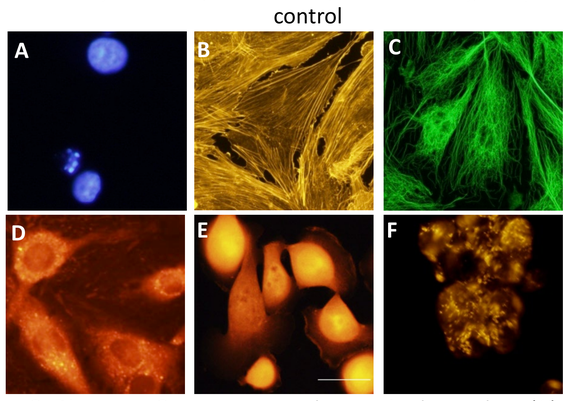

Figure 3. Discovery-Q can measure changes in the nucleus (A), actin (B), microtubules (C), mitochondria (D), cyotoplasm (E), Golgi bodies (F)

Introduction

Chemotherapy plays a major role in breast cancer treatment. However, not every chemotherapeutic is appropriate for each cancer. The mechanism of resistance to the drugs and the mechanism of action determines which patient should get which treatment. Broadly, cancer is divided into two stages: benign and metastatic. Depending on stage and other factors, each patient's cancer can be treated by any combination of chemotherapy drugs, surgical procedures, or radiotherapy. However, resistance to some drugs and general efficacy of chemotherapeutic drugs are not determined or observable until months after the start of treatment. If the patient displays chemoresistance to the drug, time spent on that therapeutic program is time wasted. A device that can test the efficacy of each drug before the treatment plan would be beneficial to the patient. Discovery-Q is such a device.

In this study, to represent benign breast cancer tumors, MCF-7 and Sk-Br-3 cell lines were used. This study tests two classes of commercially available chemotherapeutic drugs with opposing mechanism of actions. The aim is to use the Discovery-Q to be able to detect the differences in the cells' response to the drugs. The Discovery-Q is used to detect subtle differences in tumor cell proliferation post-treatment. This system provides unique kinetic information regarding the phenotypic change in the cells post treatment.

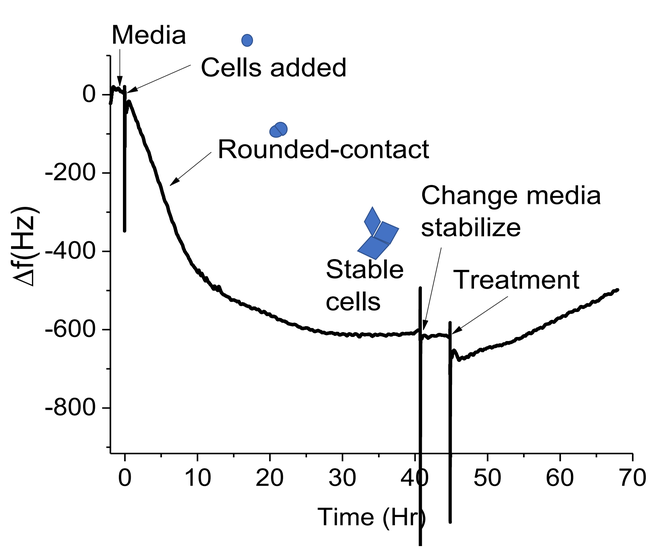

Figure 4. The standard operating procedure (SOP) for working with cells in the Discovery-Q. Data acquisition is started and media is added. Two hours are needed for protein deposition and temperature stabilization of the system. This becomes the baseline of the experiment to determine cell binding. Cells are added and allowed to bind until stabilized. A second media change occurs just before treatment. Time of treatment is used as T_0.

Methods

The MCF-7 or SK-Br-3 cell line was plated into an Discovery-Q system at 100,000 cells per well. After 24-to-30 hours, the trace was checked to ensure the cells had reached a stable baseline (Fig 4). The media was refreshed to replenish the nutrients before treatment (Fig 4). The cells were treated with varying doses of Nocodazole (0µM, 6 µM, 15 µM, and 50 µM) shown at time 0 in figure 5. The change in frequency (Δf) and change in resistance (ΔR) data was acquired over 48 hours before the experiment was completed (Fig. 5). The cells were then harvested and a cell count was performed to determine survival (Fig. 7). The experiment was repeated 3 times and the traces and cell counts were averaged (Fig 5 & 7). The times of interest were in sudden increases or decreases in the Δf; changes ΔR that were not present in the Δf; or time points where the treatment returned to the untreated control baseline and then deviated again.

Using those times, the experiments were replicated in Lab-Tek II culture slides and the cells were fixed at the time points of interest. Fluorescent imaging was used to capture the mechanisms of action causing the changes in the traces (Fig 6). The cells were triple stained to image the nuclei, Golgi bodies and microtubules. The Golgi bodies and microtubules were stained using antibodies, and in some wells, the primary antibody was omitted to check for non-specific staining to ensure that there was no cross reactivity. The stain for the nucleus was 4',6-diamidino-2-phenylindole (DAPI). The microtubule antibodies were: primary antibody mouse anti-αtubulin, and secondary antibody Alexa Fluor® 488 goat anti-mouse IgG1 (γ1). The Golgi antibodies were: primary antibody GRASP65 Polyclonal Antibody, and secondary antibody Alexa Fluor 555 donkey anti-rabbit IgG.

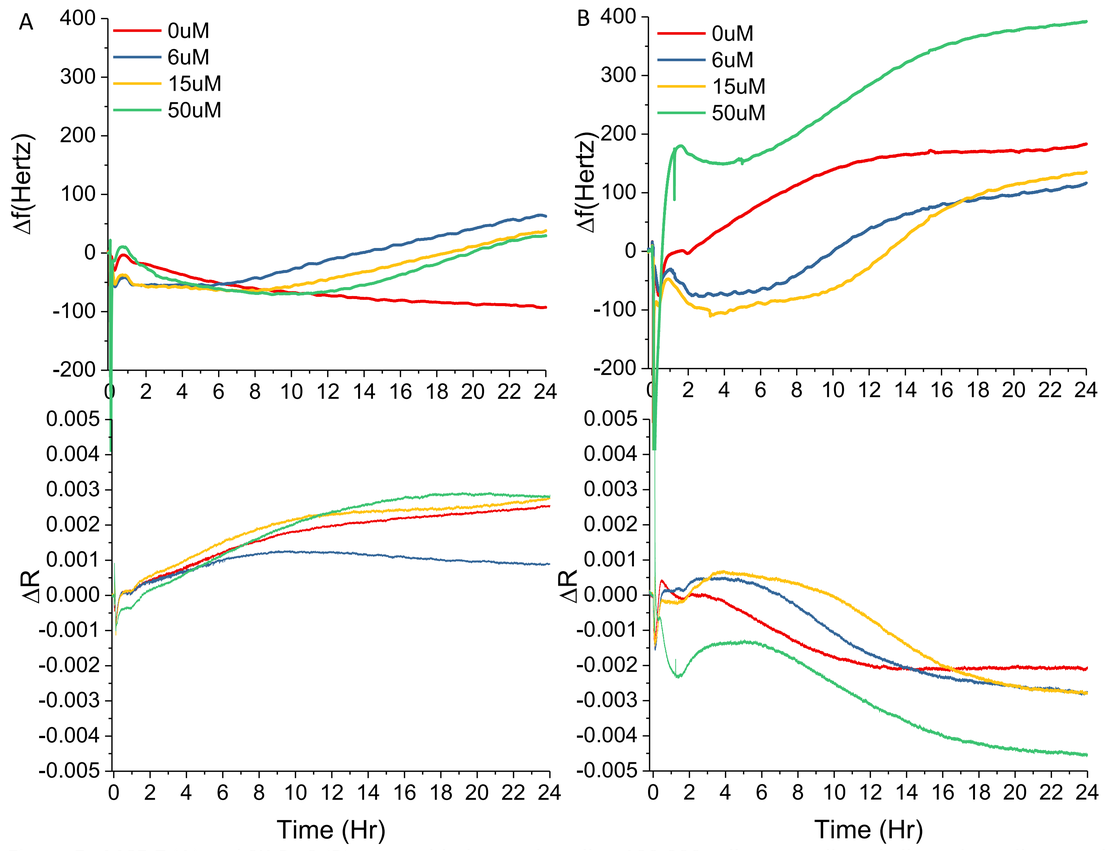

Figure 5. MCF-7 (A) and SK-Br-3 (B) were added to each well at 100,000 cells-per-well, and allowed to adhere and stabilize for approximately 24 hr. The media was changed 2 hr prior to treatment, and at time 0 (T_0) varying doses of Nocodazole were applied to the wells. Data was acquired for 48 hr. The real-time data generated illustrates the unique phenotypic signatures of breast cancer cells in response to a class of chemotherapeutics. The traces then dictate time points at which visualization should occur, and guides the elucidation of the mechanism of action. The top row is change of frequency (Δf) which corresponds to mass, and the bottom row is change of resistance (ΔR) which corresponds to cell viscoelasticity or sway.

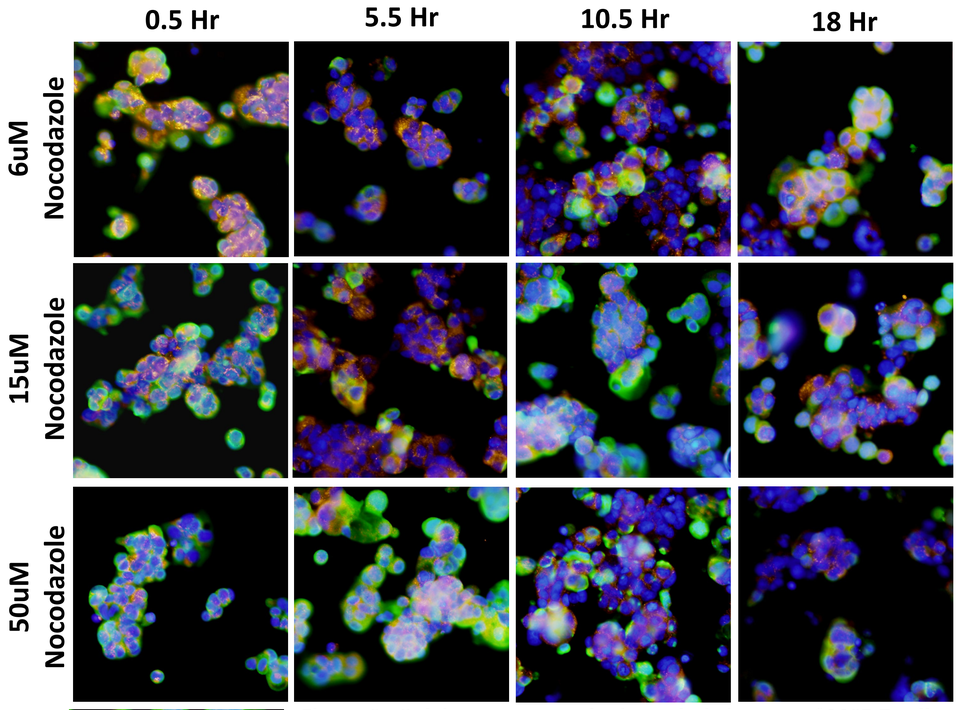

Figure 6. Golgi apparatus and microtubule images of MCF-7 cell line. Over time, and as the dosages of nocodazole increase in concentration, the microtubules round up in morphology. Microtubules begin to decrease significantly by the end of 18 hr at 50 mM of treatment. The nucleus is stained blue with DAPI, the microtubules are stained green with Alexa Fluor® 488, and the Golgi apparatus is stained red with Alexa Fluor® 555. Exposure times: DAPI, 3 ms; GRASP65, 103 ms; anti-α- Tubulin, 100 ms.

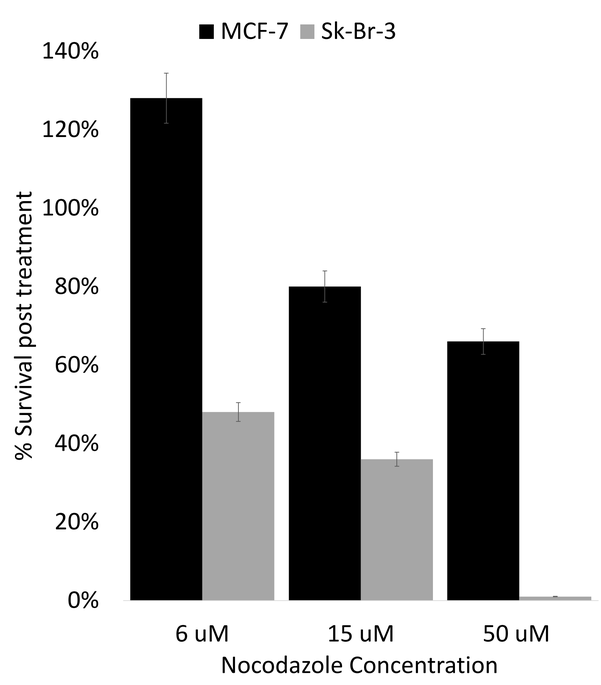

Figure 7. Percentage of cell survival after 48hr of treatment of Nocodazole. MCF-7 and Sk-Br-3 cells were treated with varying doses of Nocodazole. Overall, MCF-7 cells show higher survival percentages post-treatment of Nocodazole at all three concentrations. As a result, a conclusion can be made that MCF-7 cells display resistance to Nocodazole compared to Sk-Br-3 which experiences total cell death at 50 µM 48 hours post treatment.

Discussion

As shown in Figure 5, post treatment with nocodazole, an increase in change in resistance and a simultaneous increase in the change in frequency is not common in an Discovery-Q data set. In pervious experiments this type of change has only occurred when a subcellular organelle was perturbed. In this instance, the hypothesis as to why this occurred between 12-to-18 hours is that the Discovery-Q is detecting a change in the Golgi apparatus. According to the literature, once the microtubules begin to depolymerize, the Golgi apparatus, which is held together by the microtubules, dissociates into minisacks [1]. If the microtubules remain depolymerized, the minisacks subsequently dissolve into the cytosol [1]. Ongoing studies supported this hypothesis. Using a wash-out of the Nocodazole treatment – 10hr post addition – has shown microtubules re-polymerize and allow the Golgi apparatus to re-form. This is observed by a shift in the trace towards the untreated control over time. However, adequate repeats of the experiment are still pending. Another way to test the hypothesis would be to use a chemically active agent that directly affects the Golgi apparatus in the same manner and check to see if the Discovery-Q shows a similar response curve to the drug as the one shown in Figure 5.

After looking at the difference in cell response to Nocodazole between the Sk-Br-3 cells and MCF-7 cells, there is a clear differentiation and a conclusion can be made. Compared to Sk-Br-3 cells, MCF-7 cells show a partial resistance to the chemotherapeutic. Nocodazole has a higher efficacy in Sk-Br-3 cells than in MCF-7 cells, as shown by the increased frequency change in figure 5. When the cell viability data is also observed in figure 7, the percentage of survival post treatment was significant in comparison to the SK-Br-3. In application, if a patient's biopsy sample expresses a similar response curve to the Sk-Br-3, then an oncologist has clinically actionable information regarding Nocodazole potential drug as a chemotherapeutic with the highest efficacy.

This series of experiments has shown that the Discovery-Q as a research device can provide data that is quantitative in nature, and corresponds directly to the biomechanical changes in the cell that are not detectable by microscopy until 5+ hours post-treatment. In Figure 6, microscopically the changes that are noticeable only occur hours after activity has started at a subcellular level and cannot be quantified as thoroughly. For example, at time points 0.5 hour and 5.5 hour resemble the control (Fig 6), yet at those time points in the response curve, as depicted in Figure 5, there are obvious changes occurring.

Conclusion

The long-term goal for this project is to couple the pathologist report and genomics report of a patient's biopsy with a list of which drugs show highest absorption, retention, and potency. This could guide an oncologist to prescribe a treatment plan that is most effective, minimizing the beginning period treatment when the oncologist monitors to see if the patient's cancer will actually respond to the treatment. The next phase for this project include introducing other types of cancer like lung and colorectal cancer.

References

1. Pfeffer, S.R., How the Golgi works: A cisternal progenitor model. Proceedings of the National Academy of Sciences, 2010. 107(46): p. 19614-19618.
2. Thadani-Mulero, M., D.M. Nanus, and P. Giannakakou, Androgen Receptor on the Move: Boarding the Microtubule Expressway to the Nucleus. Cancer Research, 2012. 72(18): p. 4611-4615.
3. Crown, J., M. O'Leary, and W.-S. Ooi, Docetaxel and Paclitaxel in the Treatment of Breast Cancer: A Review of Clinical Experience. The Oncologist, 2004. 9(suppl 2): p. 24-32.
4. Blajeski, A.L., et al., G(1) and G(2) cell-cycle arrest following microtubule depolymerization in human breast cancer cells. The Journal of Clinical Investigation, 2002. 110(1): p. 91-99.
5. Chabin-Brion, K., et al., The Golgi Complex Is a Microtubule-organizing Organelle. Molecular Biology of the Cell, 2001. 12(7): p. 2047-2060.