High content in vitro cell monitoring effects of adjuvant chemotherapy in breast cancer and cancer treatment-related cardiomyopathy

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Introduction

Impedance changes of cell-covered electrodes give profound insight into cell proliferation and contractility, even over prolonged time periods, providing significant advantage over standard mass anaplastic cytotoxicity assays. Here, this technology was used for monitoring murine breast cancer cell growth after chemotherapy treatment in vitro. As the emerging field of cardio-oncology aims to find a balance between oncologic efficacy and reducing adverse cardiovascular effects, we tested the same treatment on induced pluripotent stem cell derived cardiomyocytes (IPSC- CMs). One of the standard clinical regimens for breast cancer is a combination of cyclophosphamide, Adriamycin (doxorubicin) and S-Flouranracil (CAF) administered for 4 months. Even though initially successful, tumor recurrence after this therapy remains a major cause of mortality in breast cancer patients, leaving the need for better treatment.

Long-term cell monitoring with CardioExcyte 96

The CardioExcyte 96 is a hybrid system combining on-demand and ETP (Extracelluar Pulse Potentiometry) impedance monitoring. System operates using 96 well plates with high throughputs of > 1 MPP, high-quality consumables and controlled temperature and environment make this system ideal for short and long-term cell monitoring applications such as proliferation and toxicity assays.

The CardioExcyte 96 comes with an automated liquid handling system for cell seeding, compound applications and medium exchange.

Orthotopic Tumor Model

Tumor dissociation and cell isolation revealed eleven murine HNB5 mammary carcinoma cell lines variants from 3 different groups. The murine HNB5 cells represent an immortal mammary carcinoma cell line with tumor stem cell properties. HNB11 cells represent untransformed HNB5 tumors, HNB12 cells represent rhabdomyosarcoma of the HNB5 T2 cells, and HNB13 cells represent a rhabdomyosarcoma of the HNB5 T2 cells. Tumor dissociation and Annexin V staining revealed eleven murine HNB5 and HNB12 cells were invaded via an impedance-based cell monitoring system (CardioExcyte 96) and an immunogenic breast cell analysis system (BioCarta). The impedance signal recorded on the CardioExcyte 96 changes as a result of alterations in cell conductivity, cell contact morphological shape and density of adherent cells and thereby provides a measure of cell proliferation and toxicity.

In vivo tumor growth monitoring followed by in vitro impedance recordings

Tumor growth was followed up to 8 months by using murine HNB5 mammary carcinoma cells from three different groups. The murine HNB5 cells represent an immortal mammary carcinoma cell line with tumor stem cell properties. HNB11 cells represent untransformed HNB5 tumors, HNB12 cells represent rhabdomyosarcoma of the HNB5 T2 cells, and HNB13 cells represent a rhabdomyosarcoma of the HNB5 T2 cells.

In this study, responses from murine HNB5 and isolated HNB5 cell variants were investigated by using the CardioExcyte 96 system. HNB5 T1 cells were established from a solid breast tumor that received no CAF. HNB5 T2 and T3 cells were established from a solid breast tumor that received the CAF regimen in vivo. For further in vivo tumor recurrence investigation, the HNB11 and HNB12 T1 cells were treated with CAF for the first and the HNB5 T2 and T3 cells were treated for a second time (concentrations of C: 0.020 μg/ml up to 20 μg/ml, 10.0 μg/ml up to 10 μg/ml and 0.020 μg/ml up to 20 μg/ml). The impedance-based measurement allowed monitoring of cell viability over a period of 14 months with a time resolution, while in vivo physiological condition. Here, changes in impedance, and therefore contractility, were used as a measure of cytotoxicity. Impedance-based measurement of contractility of the standard clinical treatment regimen CAF could be identified. Furthermore, it shows the short-term contractility cycles of proliferation tumor cells could be observed in some cell lines.

Investigating therapy resistance of cancer cells in vitro

In this study, we performed a parallel in vitro study to evaluate the effects of adjuvant chemotherapy on cardiomyocytes. Paclitaxel and doxorubicin administration was performed at 0.01 μg/ml and 0.1 μg/ml, respectively. The cell membrane was monitored for 1 week using the CardioExcyte 96 system. Cellular responses were compared to their clinic correlates. Cell line characteristics of paclitaxel and doxorubicin in vitro were compared to their clinic correlates. Cell line characteristics of paclitaxel and doxorubicin in vitro were compared.

Conclusions

• The label-free CardioExcyte 96 impedance platform enables acute and chronic assessment of toxicity in a continuous fashion from living cells without the confounding effects of dyes that may affect cell function.

• System uses 96 well plates with 96 parallel sensors offering high resolution recordings (1 ms imp., 0.1 μP F). Recordings are made in a completely controlled physiological environment.

• Cell adhesion and proliferation assays such as cancer immunotherapists could be successfully performed.

• In combination with murine mammary carcinoma cells, CardioExcyte 96 provides a novel tool for investigating therapy resistance of different HNB5 breast cancer cell variants in vitro.

• Cardiotoxic effects can be reliably tested using CardioExcyte 96 impedance technology. Here, we showed the effects of paclitaxel and CAF on the Cor.4U iPSC derived cardiomyocyte (NCiardca).

• Additionally, the beating pattern of cardiomyocytes could be monitored. Concentration and time dependent electrophysiological effects were observed after administration of paclitaxel and time dependent decrease in case of doxorubicin combinations.

We thank NCiardca for kindly providing cardiomyocytes (Cor.4U) on CardioExcyte 96 preplated plates.

We thank Miltenyi Biotec for isolating HNB5 cancer cell variants from the associated HNB5 tumors.