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

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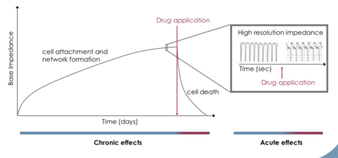
MAX-PLANCK-GESELLSCHAFT

Introduction

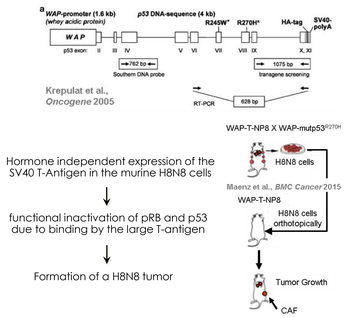
Impedance changes of cell-covered electrodes give profound insight into cell proliferation and contractility, even over prolonged time periods, providing significant advantage over standard mostly endpoint cytotoxicity assays. Here, this technology was used for monitoring murine breast cancer cell growth 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 5-fluorouracil (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 impedance and EFP (Extracellular Field Potential) recordings from the same cell. System operates using 96 well plates with high resolution sensors (1 ms imp, 0.1 ms EFP). High quality consumables and controlled temperature and environment, make this system ideal for short and long-term cell monitoring and 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.

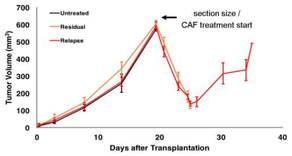


Orthotopic Tumor Model



Orthotopic transplantation of murine H8N8 cells into the syngeneic WAP-T mammary carcinoma mouse model generates triple negative breast cancer (TNBC) -like tumors similar to TNBC in the clinic. This mouse model serves as source of the murine H8N8 breast cancer cells. The murine H8N8 cells represent an immortal mammary carcinoma cell line with tumor stem cell properties.

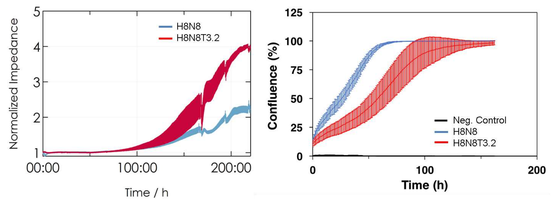
In vivo tumor growth monitoring followed by *in vitro* impedance recordings



	Animal	Tumor	Cell Line	Shortcut
Untreated	1124	6163	pH8N8T1.1	T1.1
	1194	6164	pH8N8T1.2	T1.2
	1195	6165	pH8N8T1.3	T1.3
	1214	6166	pH8N8T1.4	T1.4
Residual	1099	6172	pH8N8T2.1	T2.1
	1112	6174	pH8N8T2.2	T2.2
	1104	6173	pH8N8T2.3	T2.3
	Insufficient cell isolation			
Relapse	1098	6228	pH8N8T3.1	T3.1
	1149	6230	pH8N8T3.2	T3.2
	1106	6229	pH8N8T3.3	T3.3
	1177	6231	pH8N8T3.4	T3.4

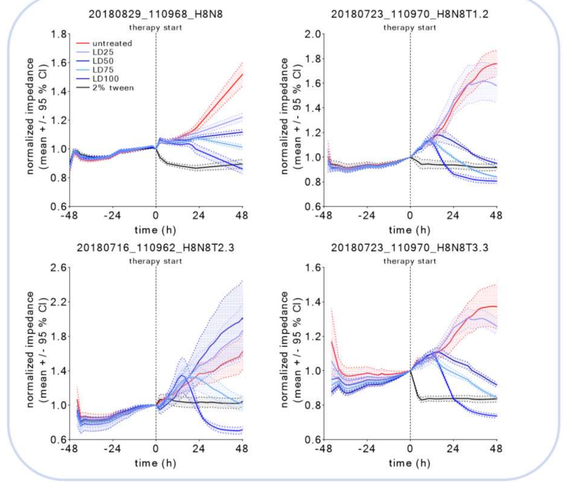
CAF Chemotherapy
100 mg/kg Cyclophosphamide
5 mg/kg Doxorubicin (Adriamycin)
100 mg/kg 5-Fluouracil

Tumor dissociation and cell isolation revealed eleven murine H8N8 mammary carcinoma cell line variants from 3 different groups. The murine H8N8 cells represent an immortal mammary carcinoma cell line with tumor stem cell properties. H8N8 T1 cells represent untreated H8N8 tumors, H8N8 T2 cells represent remitted tumors up to 9 days post CAF and the H8N8 T3 cells represent a progressive tumor variant after regrowth initiating. Cell proliferation kinetics from murine H8N8 and H8N8 T3.2 cells were investigated via an impedance-based cell monitoring system (CardioExcyte 96, left) and an image-based live-cell analysis system (IncuCyte®, right). The impedance signal recorded on the CardioExcyte 96 changes as a result of alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells and thereby provides a measure of cell proliferation and toxicity.

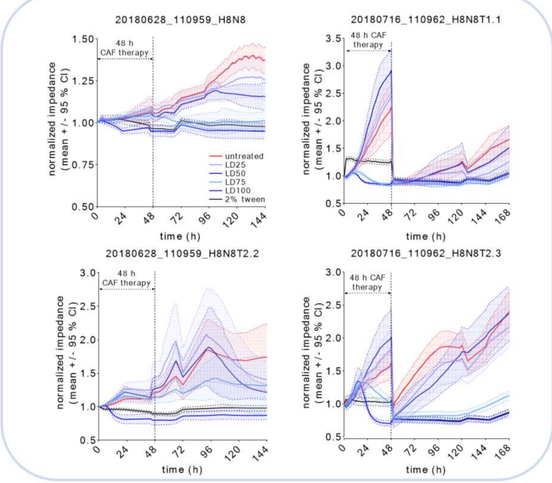


Investigating therapy resistance of cancer cells in vitro

"short term" measurement

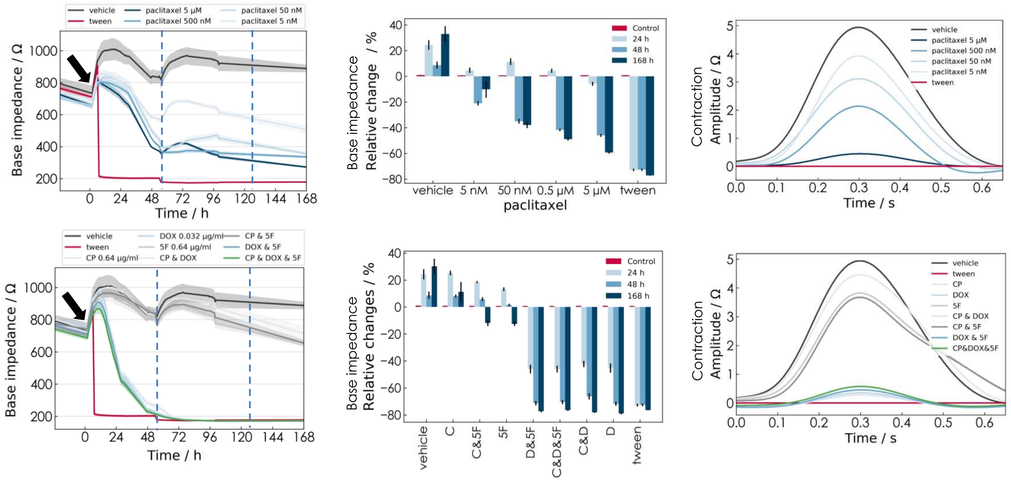


"long term" measurement



In this study, responses from murine H8N8 and isolated H8N8 cell variants were investigated by using the **CardioExcyte 96** system. H8N8 T1 cells were established from a solid breast tumor that received no CAF. H8N8 T2 and T3 cells were established from a solid breast tumor that received the CAF clinical regimen *in vivo*. For further in vitro tumor recurrence investigation, the H8N8 and H8N8 T1 cells were treated with CAF for the first and the H8N8 T2 and T3 cells were treated for a second time (concentrations of C: 0.026 µg/ml up to 20 µg/ml, D: 0.001 µg/ml up to 1µg/ml and F: 0.026 µg/ml up to 20 µg/ml). The impedance-based measurement allowed monitoring of cell viability over a time period of 168 h with a 1 ms time resolution, under in vitro physiological conditions. Here, changes in impedance, and therefore confluency, were used as a measure of cytotoxicity. Intrinsic (dose-dependent) effects of the standard clinical treatment regimen CAF could be identified. Furthermore, a dose- and treatment cycle-dependent proliferation of tumor cells could be observed in some cell lines.

Long-term cardiotoxicity monitoring using impedance



In this in-vitro study, paclitaxel and the combination of cyclophosphamide, doxorubicin and 5-fluouracil (CAF) were investigated using human iPSC derived cardiomyocytes via impedance recordings on CardioExcyte 96. Paclitaxel is a microtubule-stabilizing drug that is approved by the FDA for the treatment of ovarian, breast, and lung cancer.[1] The CAF chemotherapy regimen if often used in the adjuvant setting for the treatment of nonmetastatic breast cancer or alone for the treatment of metastatic breast cancer. However, apart from their benefits in fighting cancer, both treatments induce possible heart damage in the clinic, making cardiac toxicity an important side effect of anticancer therapies.[2] Here, we monitored the acute and chronic effects of both paclitaxel and CAF on iPSC derived cardiomyocytes (**left panels**). Black arrows indicate when the chemotheraphy solution was added. A washout with fresh medium was made after 48h (dashed lines). Cardiomyocyte´s viability and beating patterns were monitored over 190 h. Paclitaxel showed a time- and dose-dependent decrease in base impedance and impedance amplitude, cyclophosphamide and 5-fluouracil shown no or small effect, while doxorubicin shown significant toxic effects in all combinations (**middle panels**). We have also observed changes in the impedandce amplitude, here depicted as the mean beat shape change after 48 hours of treatment (**right panels**), indicating a possible effect of the treatement on cardiomyocyte contraction. [1] Weaver BA. How Taxol/paclitaxel kills cancer cells. Mol Biol Cell. 2014;25(18):2677-81.[2] Florescu M, Cinteza M, Vinereanu D. Chemotherapy-induced Cardiotoxicity. Maedica (Buchar). 2013;8(1):59-67.

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 ms EFP). Recordings are made in a completely controlled physiological environment.
- Cell adhesion and proliferation assays such as cancer immunotox assays could be successfully performed.
- In combination with murine mammary carcinoma cells, **CardioExcyte 96** provides a novel tool for investigating therapy resistance of different H8N8 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 (Ncardia).
- Additionally, the beating pattern of cardiomyocytes could be monitored. Concentration and time depent decrease was observed after administration of paclitaxel and time dependent decrease in case of doxorubucin combinations.

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

Ncardia Stem cell experts

We thank Miltenyi Biotec for isolating H8N8 cancer cell variants from the dissociated H8N8 tumors.

MACS Miltenyi Biotec