Application Forum

Gene Expression Analysis of Paclitaxel-Treated HT29 Cells Using the xCELLigence™ System and RealTime ready™ Panels

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Introduction

The analysis of gene expression profiles provides a more detailed understanding of the genetic basis of disease and supports the identification of suitable biomarkers.

Candidate compound activity and efficiency, as well as the subsequent optimization of identified lead compounds, is enhanced. Through gene expression analyses, the safety of compounds can be evaluated early in the developmental process, and the information used to avoid the risks of severe side effects.

Sensitive and specific gene expression analysis requires a concerted workflow of reliable, robust techniques. Here we describe a workflow using the xCELLigence System to monitor cellular responses of human colon carcinoma (HT29) cells after treatment with the anti-cancer agent paclitaxel in vitro, for research purposes, combined with qRT-PCR using the LightCycler®480 Instrument and RealTime ready Panels.

Based on the Cell Index profile recorded with the xCELLigence System, optimal time points were selected for the collection of samples for cell viability analysis, RNA isolation, and expression profiling.

Methods

Cell Culture and Growth Monitoring

HT29 cells were cultivated in McCoy's medium containing 10% heat-inactivated FCS in either T75 cell culture bottles for RNA isolation or an E-Plate 96 (Roche) for cell growth monitoring, and in three standard microplates for the WST-1 assay. Cells were seeded at 4000 cells/well in the E-Plate 96 and the microplates; 7.5 x 10^5 cells were seeded into each T75 cell culture bottle.

Cells were cultured for 24 h at +37°C, then 50 nM paclitaxel (in 0.0025 % DMSO) was added. Control cells treated with DMSO and untreated cells (in medium only) were monitored in parallel. All cells were incubated at +37°C. Cell growth was monitored continuously using the Real-Time Cell Analyzer (RTCA) SP Instrument (Roche) (Figure 1).

Viability Assay

Cells grown in microplates were assayed for viability using the Cell Proliferation Reagent WST-1 (Roche). One, two, and four hours after paclitaxel treatment, 10 µL WST-1 reagent was added to each well, then incubated for 1 h before absorption readout at 450 nm using a reference wavelength of 600 nm.

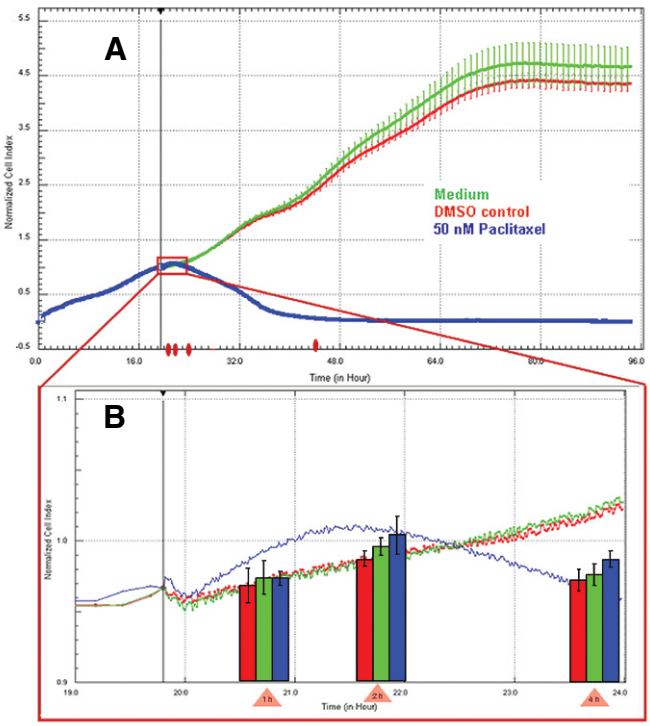

Figure 1: The xCELLigence System monitors cells in real time. Cell growth was continuously monitored using the RTCA SP Instrument. Cell Index (CI) values were normalized to the CI value at the time point of paclitaxel addition indicated by the black solid line. (A) The CI profiles of paclitaxel-treated cells (blue line), DMSO-treated cells (red line), and untreated cells (green line) reflect the initial cell attachment, logarithmic growth phase, and response to the respective treatments. The CI values for paclitaxel-treated cells approach zero approximately 24 h after treatment, indicating that nearly all cells have detached from the well bottom and are no longer contributing to the CI value. The red dots on the x-axis indicate time points selected for RNA isolation. Error bars show the standard deviation of the mean of quadruplicates. (B) Higher resolution of the CI profiles, indicating the time points selected for RNA isolation (red triangles). Colored bars represent WST-1 assay data. Color coding is identical to Panel A.

RNA Isolation and cDNA Synthesis

Cells were harvested for RNA isolation 1, 2, 4, and 24 h after paclitaxel treatment. Cellular RNA was isolated using the High Pure RNA Isolation Kit (Roche). The quality of the isolated RNA was assessed using a NanoDrop Instrument (Thermo

Scientific) and an Agilent Bioanalyzer. All samples showed high RNA Integrity Number (RIN) values (9.5–10), indicating RNA of very high quality.

One microgram of each total RNA sample was used for cDNA synthesis with the Transcriptor First Strand cDNA Synthesis Kit (Roche).

Quantitative PCR

A single cDNA synthesis reaction using 1 µg total RNA was used as template for each RealTime ready Human Apoptosis Panel, 96 (Roche). Total PCR reaction volume per well was 20 µL with the LightCycler®480 Probes Master (Roche). Sample setup and analysis were easily accomplished using the provided macro file for each gene panel with the LightCycler®480 Software 1.5.

Results

Cell Index (CI) values produced by the HT29 cells, show that the control cells at this initially seeded cell density reached confluency after approximately 70 hours (Figure 1A).

Significant changes in the CI values occurred immediately after paclitaxel treatment. Interestingly, the CI slightly increased within the first hour after treatment, before decreasing to a minimum after ~24 h. Based on this data, the first T75 bottle was harvested one hour after paclitaxel treatment for RNA isolation, reverse transcription, and qPCR. Additional samples were collected 2, 4, and 24 h after treatment.

Cell viability was analyzed with the WST-1 assay 1, 2, and 4 h after paclitaxel treatment, and resulting data were compared to the CI curves recorded using the xCELLigence System (Figure 1B). Results show that cell viability is not affected during the first four hours after paclitaxel addition.

The most significant changes in the expression levels of apoptosis-related genes occurred within the first hour after paclitaxel treatment (Figure 2). At two and four hours after paclitaxel treatment, no genes showed significant changes in expression compared to DMSO control HT29 cells.

Conclusion

The xCELLigence System records cellular events in real time without exogenous labels, providing quantitative information about the biological status of the cells, including: cell number, spreading, proliferation, and morphology. The xCELLigence System thus provides continuous surveillance of cellular changes in vitro following a given treatment, while cellular integrity is maintained.

A standard single end point cell viability assay such as the WST-1 assay will not detect changes in cell morphology and adhesion. Based on the CI profile data, we were able to determine optimal time points to perform qRT-PCR

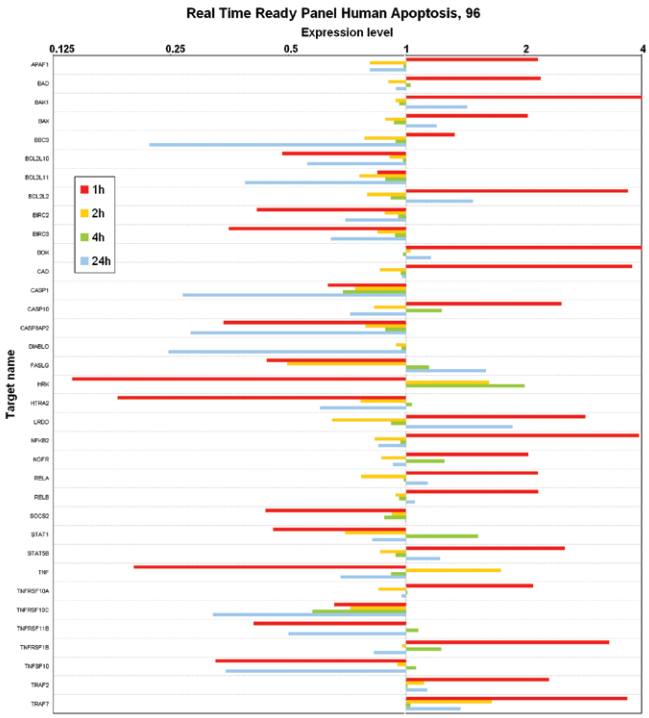

Figure 2: qPCR analysis using the RealTime ready Human Apoptosis Panel, 96 demonstrates that the crucial time point for gene expression analysis is 1 h after paclitaxel treatment. The ratio of ΔCp values of paclitaxel-treated cells compared with ΔCp values of control cells was calculated and plotted for those genes which show at least a two-fold change in gene expression compared to the control for each of the four time points. Gene names corresponding to the abbreviations can be found at **www.roche-applied-science.com/pack-insert/5392063a.pdf.**

assays, clearly demonstrating that the xCELLigence System is a powerful complement to other methods, enabling more in-depth cellular and gene expression analysis.

RealTime ready Panels are excellent tools for quantifying expression of human pathway-specific genes during cell proliferation and apoptosis. Information about pathways, genes, and assays, with links to free online databases is provided at **www.realtimeready.roche.com**.

Our data demonstrate that the combination of real-time measurement of cellular growth with subsequent qRT-PCR at selected time points identified by cell monitoring can provide significant insight into how and when paclitaxel affects cultured human carcinoma cells.

Further details of this experiment and comprehensive information about the xCELLigence System is available at **www.xcelligence.roche.com**. To learn more about other Roche Applied Science products, visit **www.roche-applied-science.com**.

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