xCELLigence System Real-Time Cell Analyzer

Focus Application
Irradiation-Induced Cytotoxicity

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Featured Study: Monitoring of Irradiation-Induced Cell Damage using the xCELLigence System

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Introduction

Exposure to γ-rays causes severe damage to essential biomolecules, including DNA double strand breaks due to direct ionization or free radical-mediated attack. Secondary injury by γ-radiation is caused by the generation of reactive oxygen species (ROS), such as oxygen radicals, triggering a cascade of free radical-mediated chain reaction. Irradiation-induced cell damage along with the generation of oxidative stress may exceed the capacity of cellular defense systems engaged in combating the basal insults encountered under normal environmental influences (1). Although exposure to γ-irradiation leads to elimination of irreversibly damaged cells by fail safe mechanisms, repeated exposure has been reported to increase the likelihood of cancer in human subjects due to an accumulation of mutations in the genome (2). The investigation of irradiation-induced cellular response and the underlying molecular mechanisms, including innate oxidative stress response, as well as DNA repair pathways (3), is a main subject of current research. To this end, we investigated the cellular response to γ-irradiation using the impedance-based xCELLigence System, allowing continuous and online monitoring of cell proliferation and cytostatic/cytotoxic effects.

Materials and Methods

HEK-293 cells were cultured in a standard humidified incubator at +37°C and 5% CO_2. DMEM cell culture medium was supplemented with 10% FCS, 2 mM L-glutamine, and PenStrep (50 U/ml penicillin G, 50 µg/ml streptomycin). Cells were harvested by trypsinization and cell number was determined using a hemocytometer. Cellular parameters, such as proliferation, viability, and cell death, were monitored continuously using the xCELLigence System. To this end, 5,000 to 20,000 HEK-293 cells were plated per 96-well on an E-Plate 96 before and after irradiation. The γ-irradiation was performed in a Gammacell® 40 Exactor Low Dose-Rate Research Irradiator (Best Theratronics) with a Cesium-137 source (1.1 Gy/min). To assess the feasibility of irradiating cells directly inside the E-Plate 96, cells were consecutively plated into the E-Plate 96 and placed back into the irradiator, accumulating a γ-ray exposure from 1 to 50 Gy. As a reference control, trypsinized HEK-293 cells were irradiated in BD Falcon tubes prior to plating. The cell damage, caused by γ-irradiation, was monitored by recording Cell Index (CI) values at 15 minute intervals for 96 hours using an xCELLigence RTCA SP Instrument.

Results

To assess the possibility of irradiating cells directly on the E-Plate 96, HEK-293 cells were consecutively plated into the E-Plate 96 and transferred repeatedly into the irradiation chamber. As a reference, trypsinized cells were irradiated in BD Falcon tubes and plated into the E-Plate 96 after treatment. With both procedures, the CI values remained the same without showing significant differences in the recorded CI profiles (see Figure 1). To monitor the cellular response of HEK-293 cells to ionizing radiation in more detail, cells were treated with increasing doses of γ-irradiation (1 – 50 Gy). Subsequently, different cell

numbers were plated in an E-Plate 96 and continuously monitored using the xCELLigence System. The CI values of irradiated HEK-293 cells decreased in a dose-dependent manner from 1 - 10 Gy, independently from the plated cell number (Figure 2). A further increase of the ionizing radiation to 50 Gy did not cause an aggravation of the observed cell damage. Treatment with 1 Gy caused a slight decrease of CI compared with non-irradiated cells, yet the CI reached control levels with a delay of 5 – 10 hours.

In contrast, the CI value of cells, irradiated with 5 Gy, stabilized 32 - 48 hours after treatment at a significantly lower CI value. The CI values of cells treated with higher doses of γ-irradiation (10 – 50 Gy) decreased to basal level between 48 and 96 hours after treatment. The irradiation-induced toxicity was quantified by calculating the inhibitory concentration 48 hours after irradiation, yielding an IC_{50} and IC_{90} of 2.4 Gy and 5 Gy, respectively.

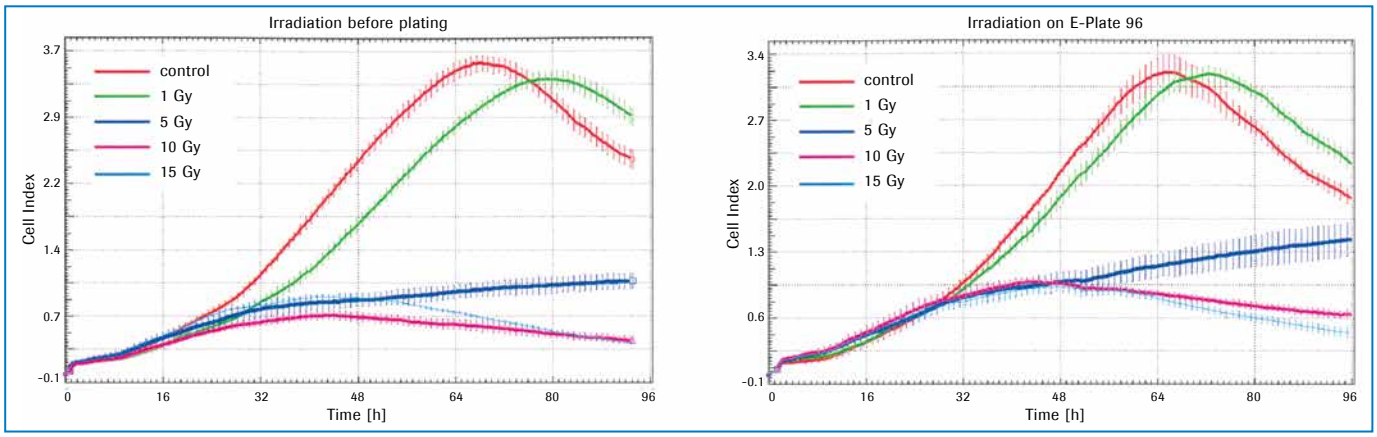

Figure 1: Assessment of γ-irradiation on E-Plates 96. HEK-293 cells were exposed to increasing doses of γ-irradiation and continuously monitored using the xCELLigence System. Cells irradiated before plating (left panel) and cells irradiated directly on the E-Plate 96 (right panel) showed equal cytotoxic effects in a dose-dependent manner.

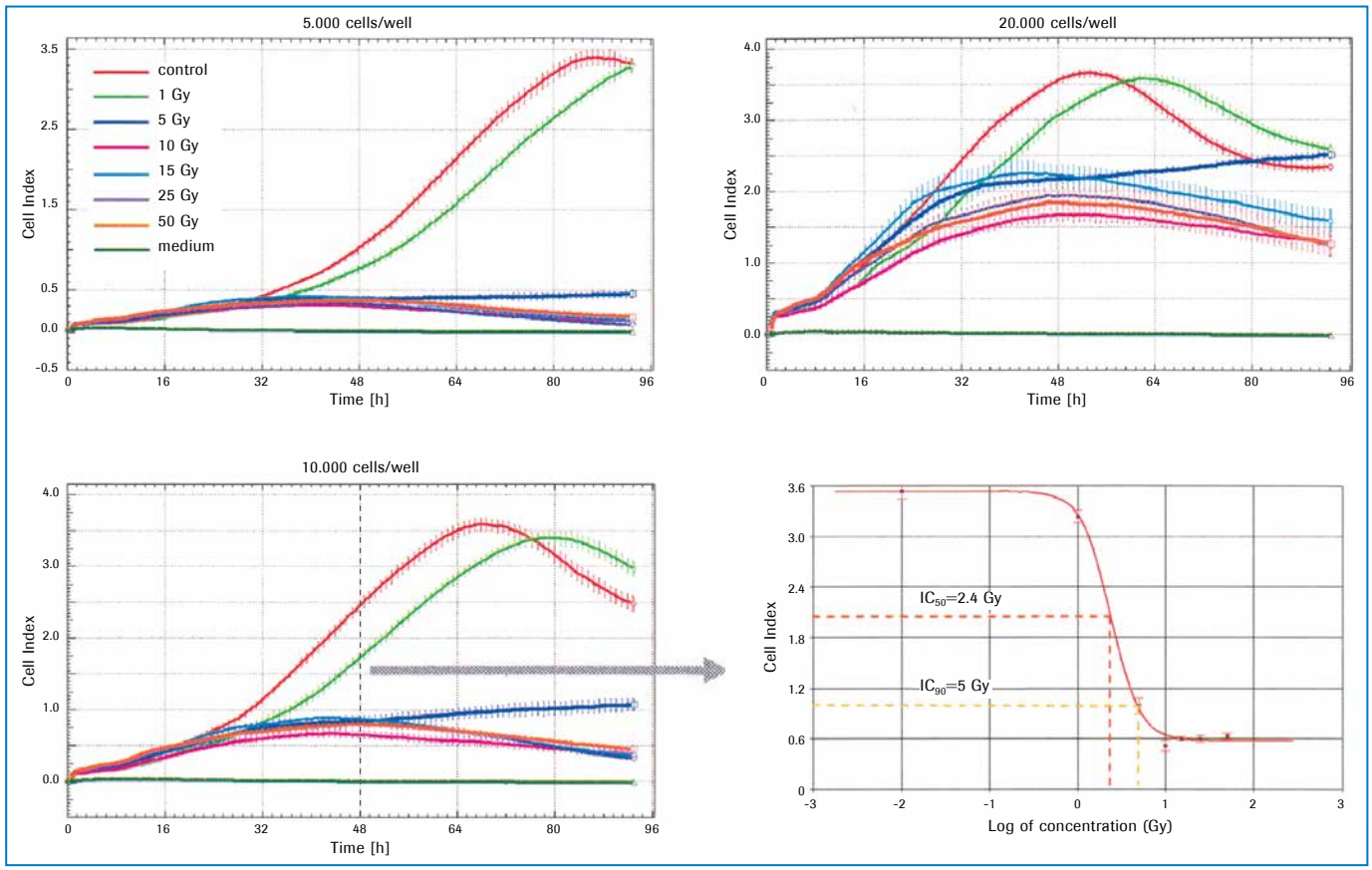

Figure 2: Continuous monitoring and quantification of irradiation-induced cytotoxicity. Different cell numbers of γ-irradiated HEK-293 cells were plated on an E-Plate 96 and cellular effects were monitored using the xCELLigence System. Cellular death occurred in a dose-dependent manner, whereas an exposure to 5 Gy caused cellular senescence. Toxicity was quantified by calculating the inhibitory concentration (IC) from a dose-response curve 48 hours after irradiation.

Conclusion

The continuous and real-time monitoring of irradiated HEK-293 cells revealed a dose-dependent decrease of the CI, reflecting the severity of cellular damage. The cellular effects could be observed with irradiation before and after plating, showing that cells can be γ-irradiated directly in the E-Plate 96 without causing any damage to the E-Plate 96. Cells treated at low doses of irradiation (1 Gy) seem to overcome the cellular damage, reaching control CI levels with a short delay. This is most likely due to a DNA damage-mediated cell cycle arrest, which is eventually overcome after the damage is repaired by the cellular DNA-repair machinery. Five gray units (Gy) of γ-irradiation abolished continuous cellular proliferation, indicating that cells undergo irreversible senescence.

This finding is in-line with protocols for the production of feeder layers. Feeder cells are frequently produced by γ-irradiation (5 - 6 Gy) of murine embryonic fibroblasts, which are mitotically inactivated to allow co-cultivation with stem cells (4). The irradiation-induced DNA-damage triggers an irreversible cell cycle arrest without killing the cell. This effect is nicely reflected by the stable CI value, which was monitored using the xCELLigence System. Optimal treatment of feeder cells is generally evaluated by proliferation assays, such as BrdU incorporation, to insure that >90% cells are mitotically inactivated.

The calculation of an inhibitory concentration (IC) by the xCELLigence System allowed us to quantify the irradiation needed to inhibit 90% of proliferation, representing an alternative approach to assess the quality of a feeder layer. The calculated IC_{90} value of 5 Gy exactly correlates with the described protocols for feeder cell production, highlighting the versatility of this system. Doses above 10 Gy caused a decrease of CI between 48 and 96 hours after irradiation, indicating massive cell death. This effect was expected as exceedingly high doses of irradiation cause massive cellular damage to chromosomal DNA as well as other bio-molecules, such as proteins and lipids.

Since cell death begins only 48 hours after irradiation, cells presumably undergo apoptosis, however, further analysis is needed to investigate the mode of cell death in more detail. Taken together, the continuous online monitoring of HEK-293 cells using the xCELLigence System revealed detailed information about the cellular responses to γ-irradiation-induced cell damage. This information can now be used for a more in-depth analysis of the underlying molecular mechanisms, such as cellular senescence and cell death signaling, in addition to the investigation of DNA damage and repair.

References

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