Titration of cytotoxic compounds as potentially radio-sensitising agents using the 24- channel live cell microscope zenCell owl.

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Introduction:

Radiation therapy, including particle irradiation, is responsible for at least 40% of cancer cures. However, to overcome therapy failure or resistance combinatorial approaches can be beneficial.

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a two-electron reductase catalysing the reduction of quinones and thus responsible for maintaining the redox balance by avoiding increased production of reactive oxygen species. NOQ1 is quite often overexpressed in many tumours and linked to the carcinogenic processes. Due to these characteristics, NQO1 can be considered as a cancer-specific target for a combined radio-chemotherapy.

Cell culture and preparation:

Human fibrosarcoma cells (HT1080) were cultivated in DMEM 4,5 g/l Glucose supplemented with 10% FCS and glutamine.

Cells were seeded at a cell density of 20000 cells/well in high glucose medium into 24-well plates and cell imaging with the zenCell owl device was started shortly after seeding. Treatment of cells was performed 24h later in low glucose media (1.0 g/l Glucose) containing different concentrations of NQO1 inhibitor for additional 24h. After 24h incubation, inhibitor was removed by adding fresh low glucose medium and cell proliferation was monitored for additional 4 days.

Results:

In order to define the appropriate NQO1 inhibitor concentrations for the treatment of HT1080 cells, we made use of the live cell imaging capabilities of the zenCell owl and observed cellular proliferation for a certain period of time (Fig.1). In comparison to classical toxicity assays, which deliver endpoint measurements only, the zenCell owl provides the opportunity to follow the growth and health of the cells in real-time without the need of addition of exogenous markers (Fig.2).

Both, untreated fibrosarcoma cells as well as cells treated with low concentrations of NQO1 (1nM) show increasing cell numbers at high proliferation rates over 6 days, finally leading to confluency (Figure 2). In contrast, higher concentrations of the NQO1 inhibitor show a dose dependent decrease in proliferation with a 50% reduction of cell counts in the lower nanomolar range (3.3-10nM). At the highest tested concentration (330nM), the proliferation was largely attenuated and could not be rescued after removal of the inhibitor. This is in contrast to lower concentrations, where a growth delay could be observed, but the cells seem to resume proliferation at day 3 after the removal of the inhibitor.

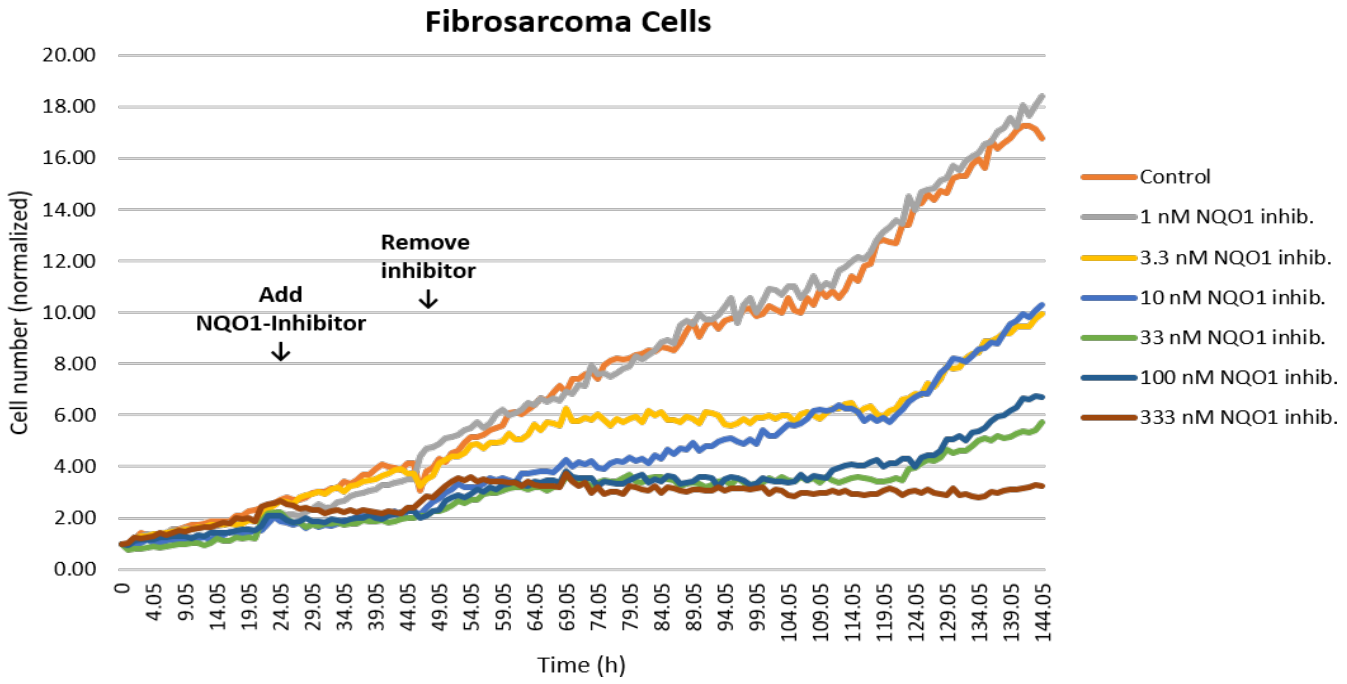

Figure 1 Analysis of cell count before, during and after treatment with different concentrations of NQO1 (NAD(P)H:quinone oxidoreductase 1) inhibitor using the zenCell owl. Fibrosarcoma cells were treated 24 h with increasing amounts of NQO1 inhibitor as indicated (arrows). After the replacement of the inhibitor by fresh medium, cell proliferation was followed for additional 4 days. Dose-dependent cytotoxicity of the inhibitor became evident by the decreased amount of cell numbers.

In addition to changes in the rate of proliferation, morphological changes in the appearance of treated cells as well as cell death can be observed and followed over time (Fig.2)

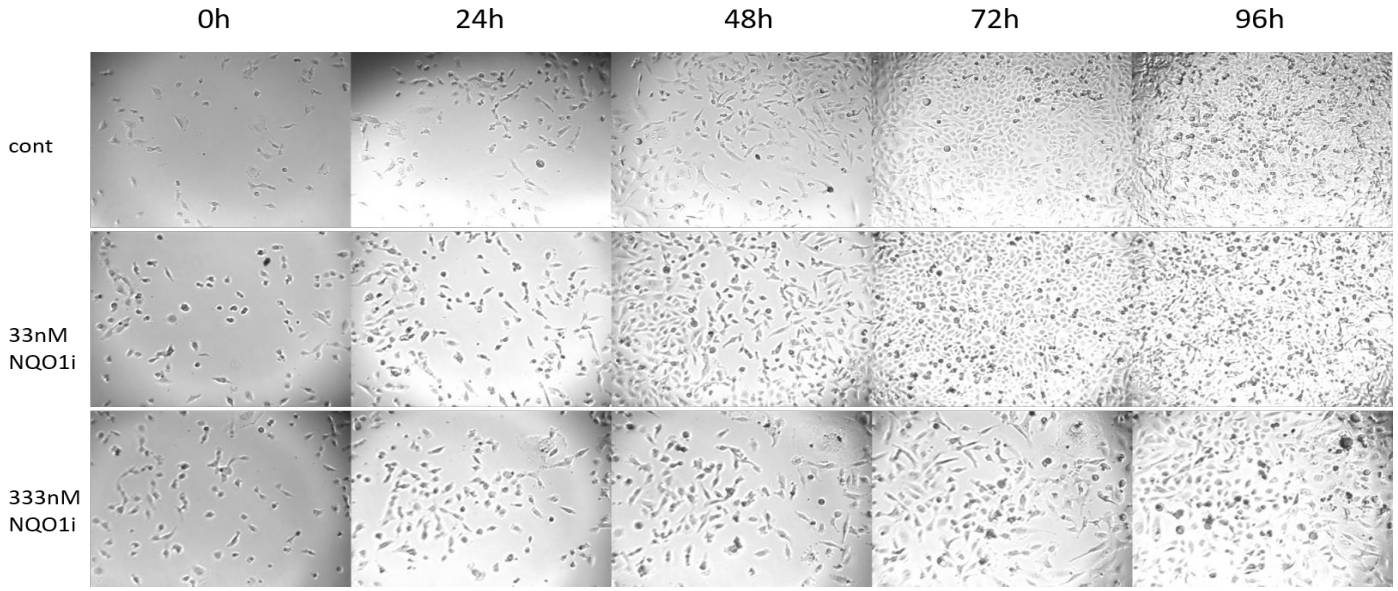

Figure 2: Cell images taken by the zenCell owl at different time points and conditions of the curves shown in Figure 1. Selected timepoints post treatment with NQO1 inhibitor are shown. Hours indicate times after addition of the inhibitor (defined as time 0h). Besides differences in cell density, reflected by the reduced cell growth in Figure 1 at higher inhibitor concentrations, cell enlargement, rounding up and loss of cells (most probably due to cell death) can be observed under treatment conditions.

Conclusion:

Depending on the cell line under investigation, the live cell imaging microscope zenCell owl is well suited to observe the time-course of cytotoxic treatments without having exogenous markers. In combination with the use of low intensity (not focused) transmitted light, photo- toxicity can be largely avoided. This allows the establishing of treatment protocols for optimal concentrations and timing in combined treatments. The 24 well format enables a simultaneous comparison of different experimental conditions.

However, the system also has some drawbacks. The illumination is not uniform in all wells leading to some artefacts. In addition, only one fix field of view per well is imaged and can be analysed. This makes the devise very small and robust, but this lowers the statistics and inhomogeneity in cell density cannot be well balanced. The software algorithm was optimized for stretched fibroblast-like cells and works well in these lines as long as they are not confluent. Other cell lines with spherical morphology and growing in dense colonies are not well recognized and separated.

However, keeping these limitations in mind, the zenCell owl is a very suitable tool in cell research.

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