Cell death and proliferation of pharmacologically relevant human primary cells with drug treatment

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Research note

Summary

It's necessary to test how new therapeutic drugs impact cell viability at an early stage of the development process. Here we present how the cytotoxic and cytostatic effects of drug treatment on different pharmacologically relevant primary human cell types can be monitored with high throughput-suitable cell-based assays. Clinically relevant side effects can be measured in a standardized, application-friendly *ex vivo* setting using normal primary cell types explanted from the human body.

We showed that treatment with experimental drugs elicits a range of responses by the primary human cell types studied. As expected, cells of epithelial and keratinocyte origin were the most sensitive to drug treatment and exhibited the highest rate of cell death. In contrast, human cardiac myocytes were the most resistant to cell death induction. The test format, involving primary human cells and standardized assays, permitted convenient, highly reproducible application in a high-throughput setting. With the use of different human cell types, this pharmacologically relevant cellular testing system comes closer to reproducing *in vivo* physiological conditions than immortalized cell lines.

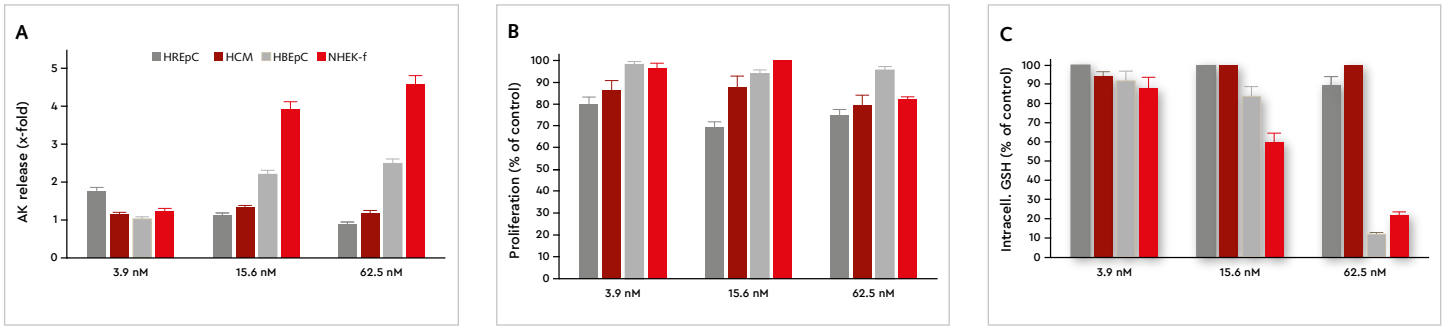

Fig. 1: Differential response of human cells to actinomycin D. *Primary human cells from renal epithelium (HREpC), cardiac muscle (HCM), bronchial epithelium (HBEpC) or keratinocytes from newborn foreskin (NHEK-f) were treated with the indicated concentrations of the chemotherapeutic drug actinomycin D for 16 hours.* ***A:*** *The release of adenylate kinase (AK) from treated vs. untreated cells was measured as an indication of cell death.* ***B:*** *The inhibition of proliferation was measured with nuclear staining of the cells.* ***C:*** *A decrease of intracellular levels of reduced glutathione (GSH) was quantified as an indicator of oxidative stress. The diagrams show the results obtained from two executions.*

Results and discussion

In order to test drug-mediated cellular toxicity, primary human cells from renal epithelium (HREpC), cardiac muscle (HCM), bronchial epithelium (HBEpC) and keratinocytes from foreskin (NHEK-f) were chosen. These cell types represent pharmacologically relevant human tissues and are therefore suitable for predicting drug-induced side effects in human subjects. To measure cytotoxicity, cell proliferation and cell/oxidative stress, we performed typical high-throughput-adaptable assays that are suited for drug toxicity/toxicology screening.

First we tested actinomycin D, a chemotherapeutic agent, on these cell types. To monitor the induction of cell death, the release of adenylate kinase (AK), an intracellular enyzme, from the cells was analyzed. After 16 hours of incubation, we only found a significant increase in AK activity in the cell supernatants in HBEpC and NHEK-f (Fig. 1, A), while HREpC and HCM were impervious to drug treatment. This indicates that two cell types underwent necrotic cell death as a result of drug treatment, while no significant effects on survival were observed in the other two cell types.

Next, we tested for cell proliferation after 16 hours of drug treatment. Interestingly, inhibition of proliferation was observed in all tested cell types, but here HREpC and HCM were most sensitive (Fig. 1, B). This result shows that actinomycin D treatment inhibits mitosis in all of the tested cell types, although sensitivity varies depending on their proliferative capacity.

Next, we investigated whether two different drugs, namely the topoisomerase inhibitor camptothecin (which is also used as a cancer therapeutic) and the protein synthesis inhibitor cycloheximide would have different effects when applied to primary human cells. After 16 hours of incubation, we measured the release of adenylate kinase (AK) from treated vs. untreated cells and found significant increases in HBEpC, HREpC and NHEK-f with camptothecin treatment (Fig. 2, A on the left), while cycloheximide only lead to AK-release from NHEK-f (Fig. 2, A on the right). These results parallel the observations found with actinomycin D treatment (Fig. 1 A) and shows that primary keratinocytes are most sensitive to cell death as a result of drug treatment.

Furthermore, under these conditions HCM did not release any AK, indicating that no necrotic cell death was induced. Again, this result is in line with our previous data on actinomycin D (Fig. 1, A), showing that myocytes are most resistant to cell death induction by drug treatment.

We then tested for cell proliferation with camptothecin or cycloheximide (Fig. 2, B). As already observed with actinomycin D (Fig. 1, B), all cell types showed some degree of inhibition, but in contrast to our previous data, HREpC now exhibited the greatest sensitivity towards both drugs. It appears that camptothecin-mediated cell death of HREpC (Fig. 2, A on the left) is associated with inhibited proliferation (Fig. 2, B on the left), while cycloheximide treatment of HREpC did not induce cell death (Fig. 2, B on the right) but robustly inhibited cell proliferation (Fig. 2, B on the right). Consequently, depending on the cell type and the drug tested, induction of cell death and inhibition of cell proliferation may be separate events.

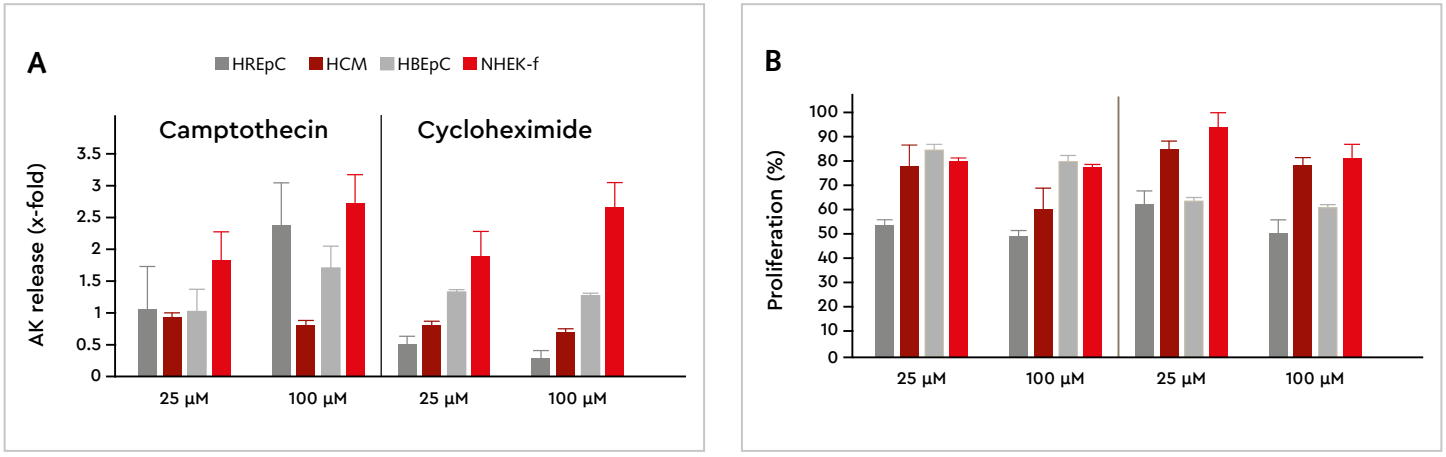

Fig. 2: Cell death and decreased proliferation of primary human cells. *Cells from renal epithelium (HREpC), cardiac muscle (HCM), bronchial epithelium (HBEpC) or keratinocytes from newborn foreskin (NHEK-f) were treated with the indicated concentrations of camptothecin (left) or cycloheximide (right) for 16 hours.* ***A:*** *Release of adenylate kinase (AK) from treated vs. untreated cells was measured as an indication of cell death.* ***B:*** *Inhibition of proliferation was measured by nuclear staining of the cells and depicted relative to untreated cells.*

Conclusion

To sum up, our findings reveal that various pharmacologically relevant primary human cell types exhibit different sensitivities to treatment with experimental drugs. The cellular responses observed, namely cell death, inhibition of proliferation and induction of oxidative stress, are cell-type-specific and reflect clinical data on the tissue-specific side-effects of drug treatment in humans. It was also easy to use human primary cell culturing with common cell-based assay formats in a high-throughput setting.

The use of primary cells from different human tissue types in the preclinical drug development process is therefore a valuable approach for monitoring toxic side effects on healthy human cells.

Materials and Methods

Primary human cells and the corresponding cell culture media were obtained from PromoCell GmbH, Germany and cultured according to the manufacturer's instructions.

The assays listed below were also obtained from PromoCell GmbH, Germany and used according to the manufacturer's instructions in a microtiter plate format (products discontinued). Cell viability was monitored bioluminometrically based on the release of adenylate kinase (AK) from necrotic cells, cell proliferation by fluorometric staining of cell nuclei, and oxidative stress by fluorometric detection of intracellular levels of GSH. The drugs were part of the Apoptosis Inducer Set (PromoCell GmbH, Germany, products discontinued).

A Spark multimode plate reader (from Tecan Group Ltd. in Switzerland) was used for the fluorometric and bioluminometric measurements on 96-well microtiter plates.

Experiments conducted by PD Dr. Rüdiger Arnold (Heidelberg)

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Products

Media	Size	Catalog number
Human Bronchial Epithelial Cells (HBEpC)	500,000 cells	C-12640
Airway Epithelial Cell Growth Medium	500 ml	C-21060
Human Renal Epithelial Cells (HREpC)	500,000 cells	C-12665
Renal Epithelial Cell Growth Medium 2	500 ml	C-26030
Normal Human Epidermal Keratinocytes (NHEK)	500,000 cells	C-12001
Keratinocyte Growth Medium 2	500 ml	C-20011
Human Cardiac Myocytes (HCM)	500,000 cells	C-12810
Myocyte Growth Medium	500 ml	C-22070
Keratinocyte Growth Medium 3	500 ml	C-20021

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