

Doxorubicin-induced Toxicity in Cardiomyocytes Derived from Human Pluripotent Stem Cells

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INTRODUCTION

Doxorubicin is an efficient chemotherapeutic agent for a variety of cancers, including leukemia, lymphomas, and many solid tumors. Doxorubicin treatment is, however, associated with severe cardiotoxicity, often resulting in early discontinuation of the treatment. The exact mechanisms involved in doxorubicin-induced cardiomyopathy are not known, but the variation in time-to-onset of toxicity and differences based on gender and age suggest that several mechanisms may be involved. New and relevant biomarkers for prediction of doxorubicin-induced cardiomyopathy will likely be of great importance.

In this study, the toxic effects of doxorubicin exposure were investigated in pure cardiomyocyte cultures derived from human embryonic stem cells (hESC). The cell morphology and contractile ability were monitored during a 48h exposure and 12 days recovery period. RNA was isolated at defined time points and in addition, Lactate Dehydrogenase (LDH) and Troponin T (cTnT) leakage from the cells were measured in the culture medium. Global gene expression was analyzed with the aim to investigate mechanisms and cellular pathways activated in the cells during and after doxorubicin treatment.

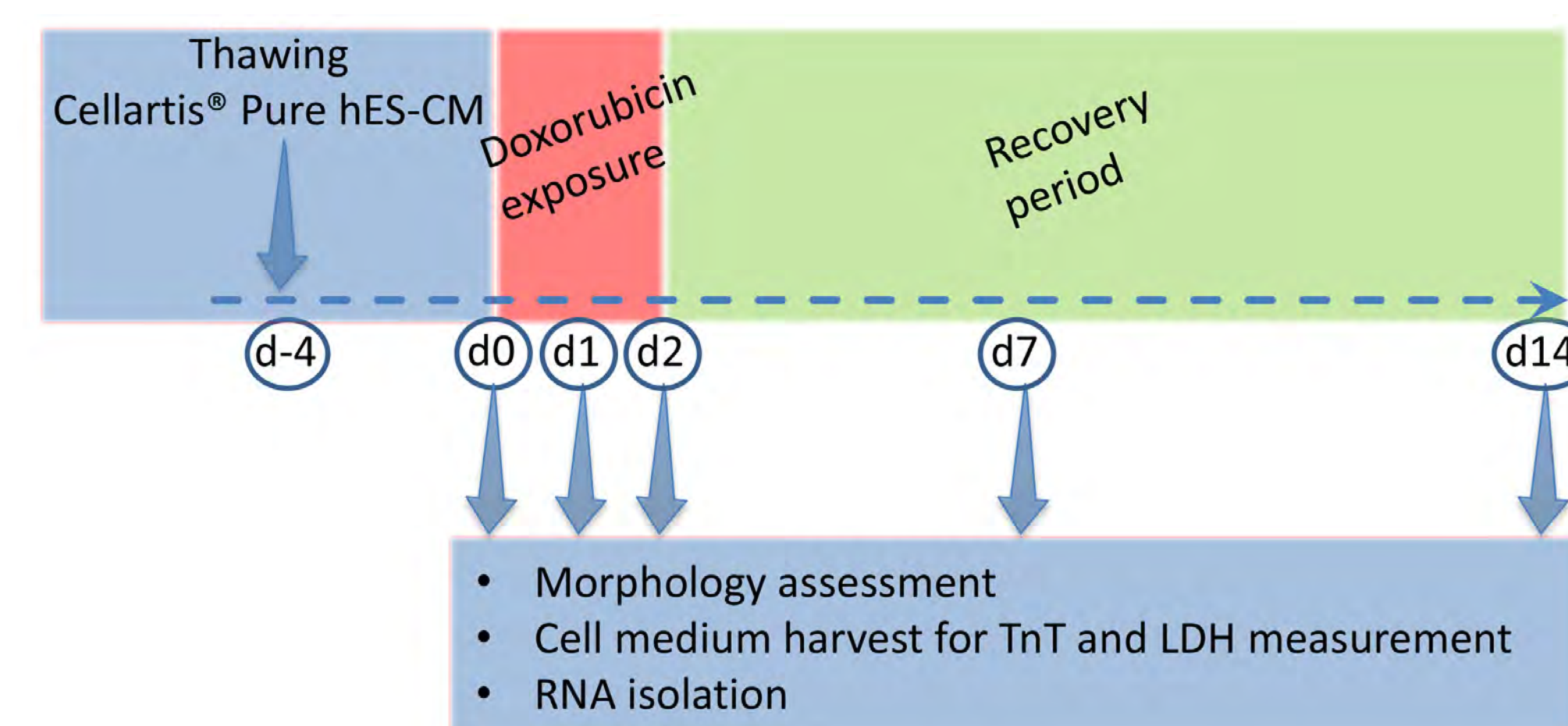


Figure 1. Study outline

METHODS

The cardiomyocytes (Cellartis® Pure hES-CM, Collectis AB) were seeded at 200 000 cells/cm² and cultured in a homogenous monolayer. Four days post-thawing, the cells were exposed to a low- (50nM), medium- (150nM), and high- (450 nM) dose of doxorubicin for 48h, followed by a 12 days recovery period.

Cells were harvested for RNA extraction after 24h and 48h of doxorubicin exposure as well as after 5 and 12 days recovery, post exposure. In addition, LDH and cTnT leakage from the cells were measured in the culture medium (fig. 1). Global gene expression was analyzed with HuGene ST 2.0 arrays from the Affymetrix platform.

RESULTS

The Cellartis® Pure hES-CM displays a homogeneous population (~90 % TnT positive cells) of physiologically relevant cardiomyocytes that show stability for long-term culture (fig. 2).

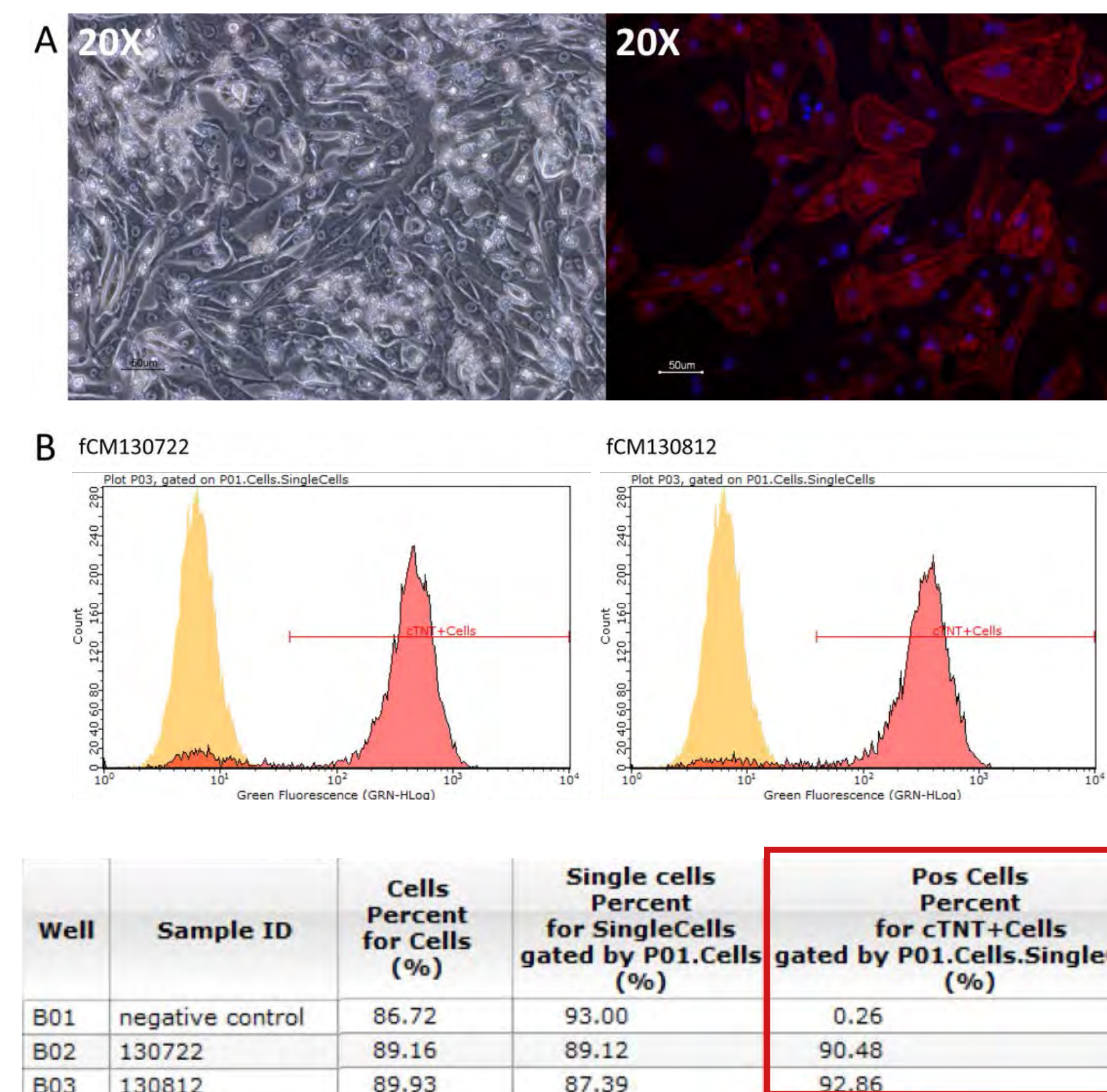


Figure 2 A. Light microscopy image and immunocytochemistry image of Cellartis® Pure hES-CM stained with cTnT and DAPI
B. FC analysis of Cellartis® Pure hES-CM stained with cTnT antibody 3 days post-thawing. Cells seeded at 200k/cm²

There is an evident effect of the doxorubicin exposure even after the recovery period. The cell morphology is altered (fig. 3) and the cells show a reduced contractile ability, most prominent in the highest concentration at the later time points.

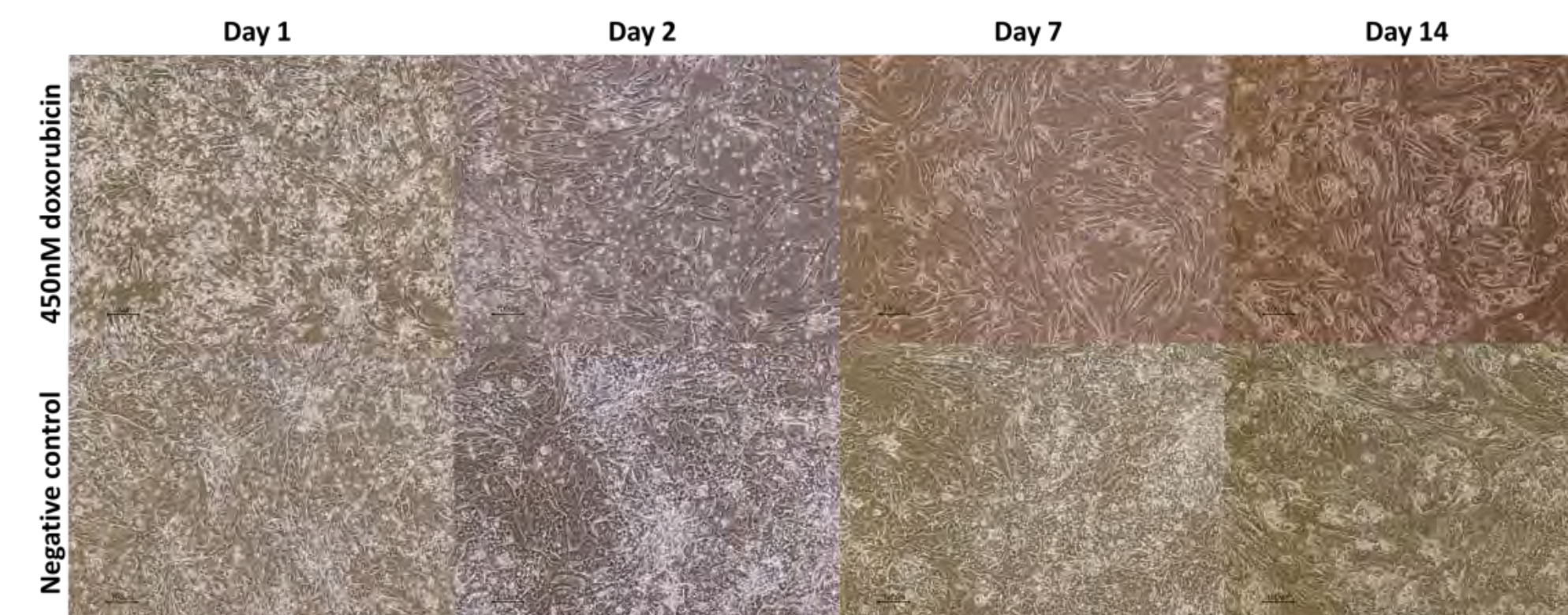


Figure 3. Light microscopy images of Cellartis® Pure hES-CM exposed to doxorubicin

A general cytotoxic response measured by LDH leakage is observed after 48h exposure compared to the vehicle control. Nonetheless, this response vanishes during the recovery period. A similar pattern is observed for the cardiac specific Troponin T release (fig. 4).

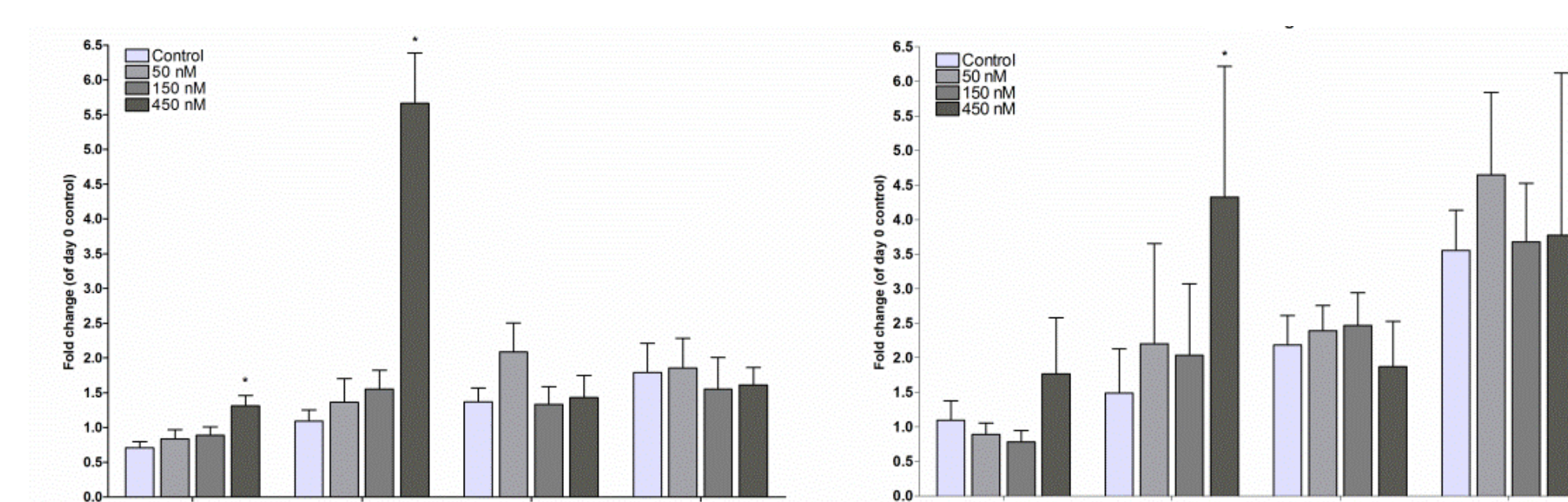


Figure 4. cTnT and LDH measurement in cell culture medium using ELISA and Lactate Dehydrogenase Activity Assay Kit, Sigma-Aldrich, respectively. Error bars = standard deviation (n=3)

A hierarchical clustering of all microarray samples indicates a similarity between the replicates and a time effect as well as a treatment effect (fig. 5).

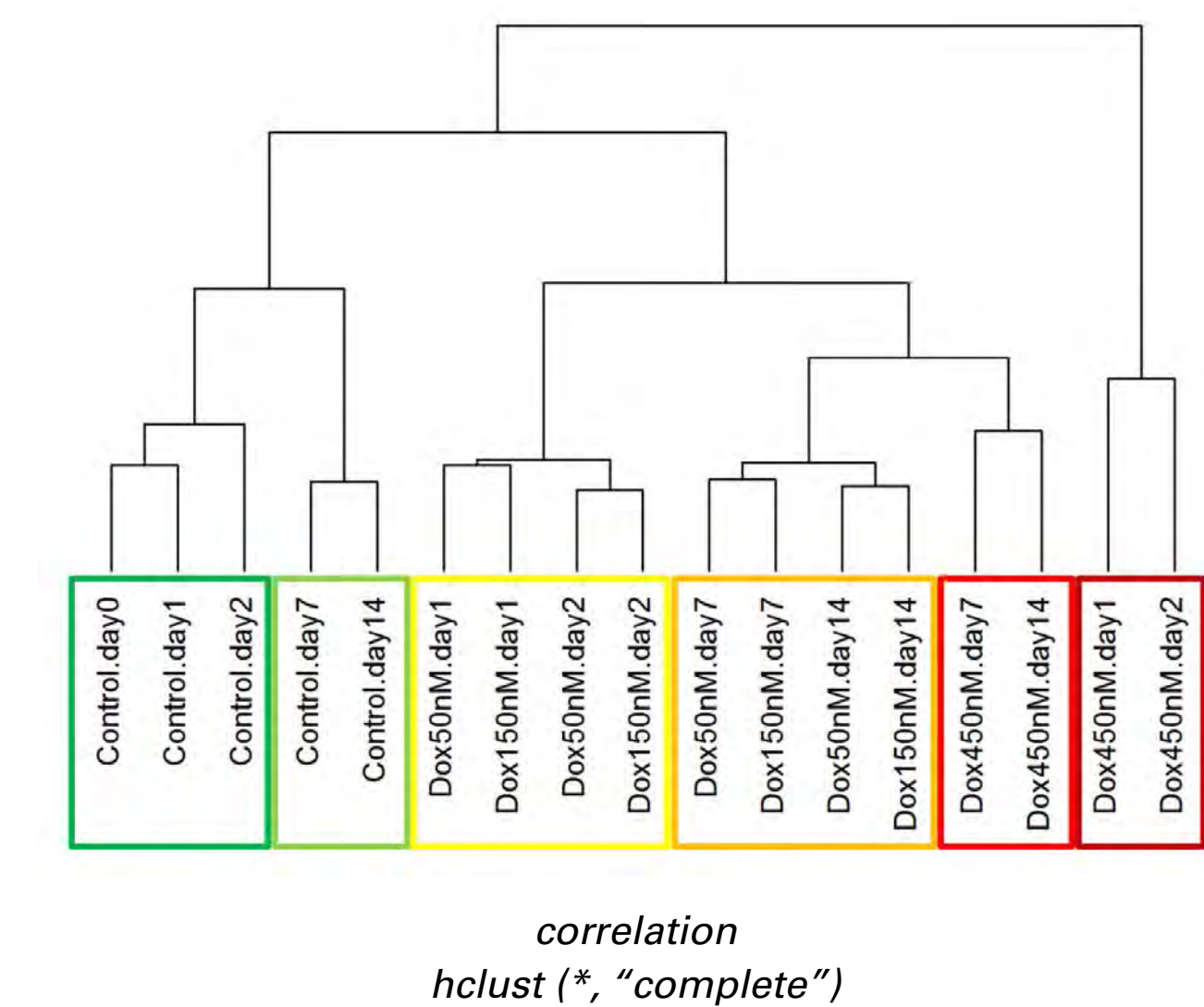


Figure 5. Hierarchical clustering of the mean values of a sub set of 21,466 transcripts display a separation of the samples into six distinct clusters

Global gene expression analysis identified several cellular pathways affected by the doxorubicin treatment. We also identified GDF-15, as well as additional genes with an interesting expression profile, with the potential to be novel biomarkers (fig. 6).

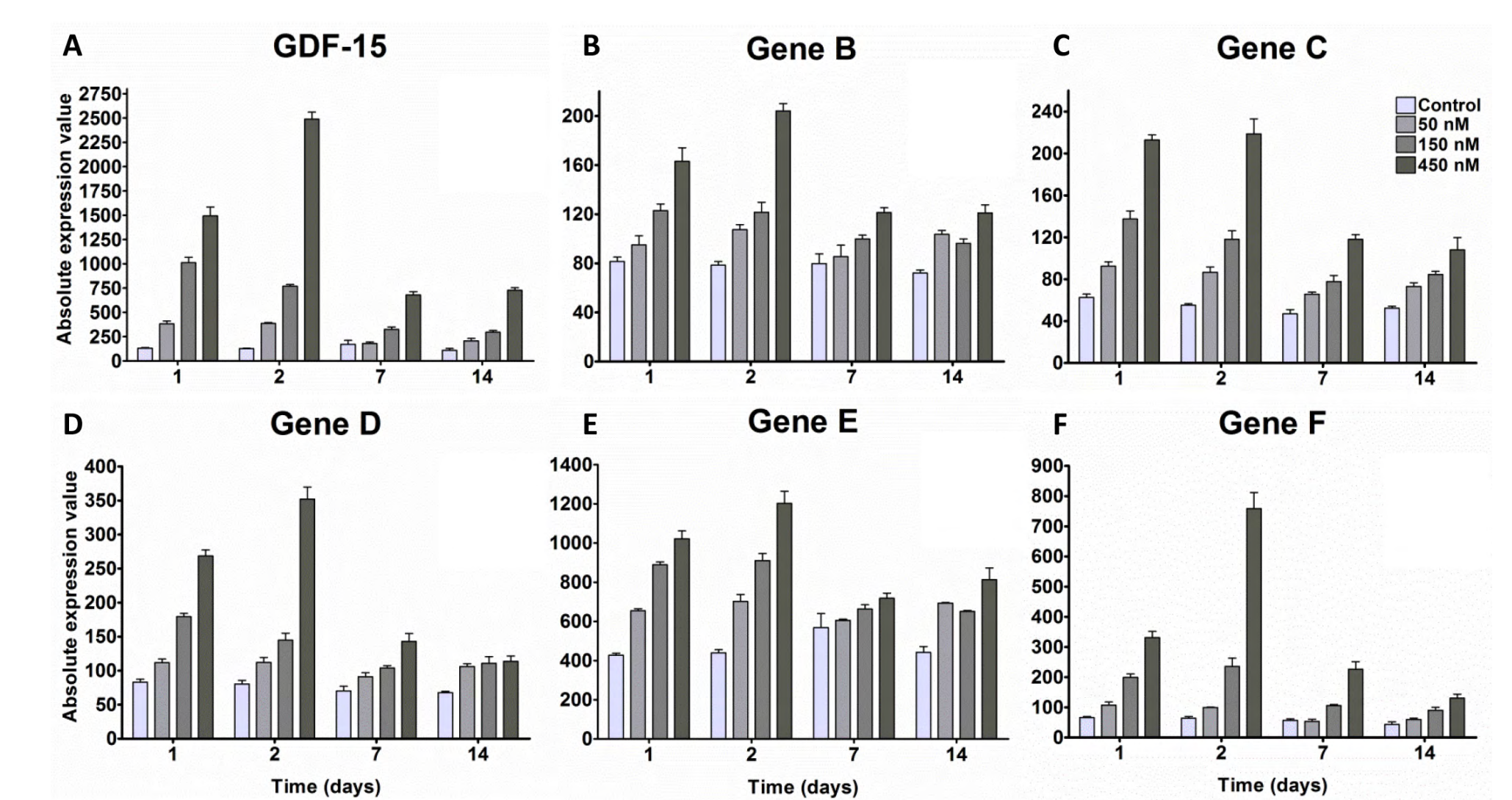


Figure 6. Expression of 6 genes with interesting expression profile that might be relevant as biomarkers for doxorubicin-induced cardiotoxicity

CONCLUSIONS

- Doxorubicin exposure shows an evident effect on the cells' morphology and contractile ability.
- The tested biomarkers (cTnT and LDH) can be a measurement of the acute cardiotoxicity due to doxorubicin exposure. However, for the late-onset cardiomyopathy they might not be appropriate.
- The gene expression of GDF-15 is a more sensitive marker compared to cTnT measurement and, as such, might be a more predictive biomarker than the conventional biomarkers used for doxorubicin-mediated cardiovascular events.
- Other genes with a similar expression profile as GDF-15 with the potential to be novel biomarkers were also identified.