Simple method rescues stressed liver cells

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Isolated human hepatocytes are essential tools in preclinical and clinical liver research, but cell quality is highly variable. Now, researchers from Uppsala University have devised a simple protocol that improves hepatocyte quality and enables cells from a wider quality spectrum to be used in standard and advanced cell culture. The findings are published in *Archives of Toxicology*.

Hepatocytes are responsible for detoxification of the blood, and constitute around 80 percent of the liver volume. They are used extensively in laboratory experiments, such as studies of drug uptake, metabolism, and toxicity. Freshly isolated human hepatocytes are not regularly available, however, as they can only be prepared by highly specialized laboratories. Therefore, researchers rely on deep-frozen (cryopreserved) cells to ensure continuous access. Unfortunately, freezing and thawing mammalian cells is very stressful and frequently results in loss of function.

"The cellular stress associated with isolation and freezing takes its toll on the hepatocytes, and many cells are too damaged to recover completely after thawing. When too many cells are damaged, they become practically useless for most applications," says Magnus Ölander, a Ph.D. student in the Drug Delivery group headed by professor Per Artursson at Uppsala University.

The research group used state-of-the-art mass spectrometry to compare the expression of thousands of proteins in damaged and healthy hepatocytes, and found that the damage involved apoptosis, a controlled

form of cell death.

"Through further analysis, we noticed that the damaged cells were mostly in the early stages of apoptosis. We reasoned that if we could figure out a way to temporarily decrease the stress, we could give the cells a chance to recover," says Magnus Ölander.

The researchers therefore treated hepatocytes with different stress-reducing compounds, and discovered that the damage could indeed be reversed by using a specific apoptosis inhibitor. Based on these findings, they designed a simple restoration protocol that improves the quality of suboptimal human hepatocyte preparations to the point where they can be used for most applications, with restored functionality in terms of drug uptake, metabolism, and toxicity. This is the first time that human hepatocytes of suboptimal quality have been 'rescued' from the freeze state, which has previously been considered a futile endeavor.

"Another novel aspect is the transient nature of our approach. The inhibitor is only used for a short time after thawing, and does not need to be included in the cell culture medium. We predict that our protocol can dramatically increase the availability of human hepatocytes of high quality, as suboptimal human hepatocytes can be found in deep-freezers in laboratories all over the world. This will ultimately give the scientific community improved access to these important cells," says Magnus Ölander.

More information: Magnus Ölander et al. A simple approach for restoration of differentiation and function in cryopreserved human hepatocytes, *Archives of Toxicology* (2018). DOI: 10.1007/s00204-018-2375-9

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