Commentary: Crocetin protected human hepatocyte LO2 cell from TGF-β-induced oxygen stress and apoptosis but promoted proliferation and autophagy via AMPK/m-TOR pathway

Ralf Weiskirchen*

Institute of Molecular Pathobiology, Experimental Gene Therapy and Clinical Chemistry (IFMPEGKC), Rhine-Westphalia Technical University (RWTH) University Hospital Aachen, Aachen, Germany

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In a recent article published in *Frontiers in Public Health*, Guo et al. investigated the therapeutic effects of crocetin on liver injury using the LO2 cell line (1).

Crocetin is a naturally occurring apocarotenoid 20-carbon dicarboxylic acid found in the crocus flower *Crocus sativus L* (Figure 1). The plant also known as saffron is used as a traditional medicine for the treatment of liver disorders (2). The health-promoting effects of crocetin and its derivatives have been confirmed in many other diseases, including hypertension, atherosclerosis, myocardial hypertrophy, arrhythmia, myocardial infarction, myocarditis, coronary artery diseases, stroke, hyperlipidemia, diseases of the nervous system, retinal damage, and many types of cancer (3, 4). This compound can inhibit the growth of cancer cells, most likely through the downregulation of genes involved in inflammation (5). Regarding the liver, it was previously shown that crocetin protects against fulminant experimentally induced hepatic failure and many other hepatic lesions by decreasing apoptosis, inflammation, and oxidative stress in rats and by inhibiting several upstream kinases in cells of hepatic origin (4, 6). In particular, crocetin showed hepatoprotective properties in carbon tetrachloride-induced liver injury in mice via induction of antioxidant defense (7). Similarly, crocetin improved Dengue
Nowadays, this cell line is not anymore available at ATCC, and the Expasy Cellosaurus database lists this cell line under entry CVCL_6926 as a “Problematic cell line: Contaminated. Shown to be a HeLa derivative,” while the register of the International Cell Line Authentication Committee (12) (ICLAC-00575) states that “L-02 was reported to be established from normal liver cells but its STR profile corresponds to HeLa” (13). Therefore, the presented data solely established with this line have to be taken with caution.

Cell line misidentification is a general serious problem in biomedical research. A conservative estimate found 32,755 articles reporting results with misidentified cells, which in turn were cited by an estimated half a million other papers (14). Another more recent estimate suggested that 8.6% of all cell lines used are problematic cell lines (15). Moreover, it is estimated that 5% of the human cell lines used in manuscripts considered for peer review are misidentified (16). At the same time, the actual ICLAC register released on 8 June 2021 lists nearly 600 cell lines known to be misidentified through cross-contamination or other mechanisms, which once again demonstrates the scale of the problem (12).

Although apparent HeLa cell contamination of human cell lines has already been known for a long time (17) and cell authentication has been widely recommended for many years, the scientific community is still not sensitized enough to recognize the dangers associated with misidentification and cross-contamination (18).

Besides L02 cells, there are many other misidentified cell lines frequently used in hepatology research (19). Like the L02 cell line, most of them have been shown by short tandem repeat (STR) analysis to be contaminated by or to be identical to HeLa. Prominent hepatic cell lines that are supposed to be misidentified or contaminated with other cell lines include Chang liver cells, GREF-X, Hul-1, WRL 68, and REPC (19). Unfortunately, several scientists still ignore the guidelines and
recommendations of the ICLAC to use these lines as derivatives of primary liver cells. However, the usage of a misidentified cell line in a study does not imperatively mean that the statements or conclusions of the study are questionable. Based on the previously mentioned literature, there is no doubt that crocetin impacts cell viability, cell apoptosis, and cell autophagy by impacting AMPK/m-TOR signaling as supposed by Guo et al. Nevertheless, the overall conclusion is that the results demonstrate that crocetin is a compound that might have therapeutic effects on the pathogenesis of a hepatic disease is not underpinned by any data from this study. Importantly, other studies have very recently shown that crocetin negatively affects bile acid formation and disturbs intestinal homeostasis by promoting inflammation and altering gut microbiota in mice (20), which contradicts the findings of Guo et al.

In sum, the study by Guo et al. would be better titled “Crocetin protects from TGF-β-induced oxygen stress and apoptosis and promotes proliferation and autophagy via the AMPK/m-TOR signaling pathway in LO2/HeLa cells.” This title would better be suited to summarize the findings of the study and avoid misinterpretation.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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