EXAMPLE OF APPLICATION

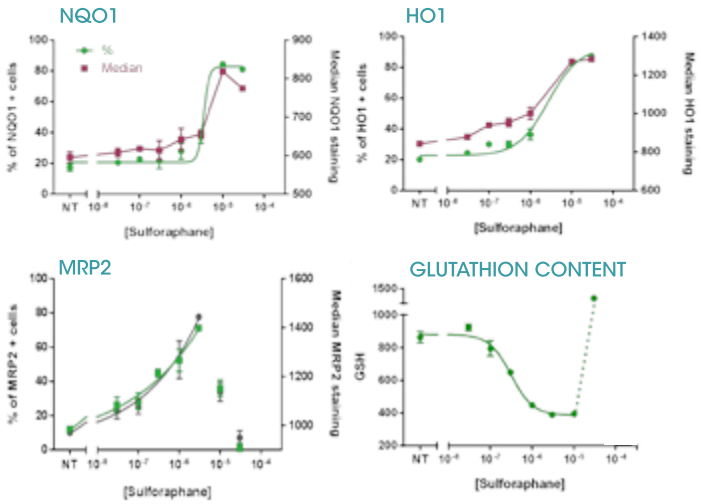
IDENTIFICATION OF HEPATOPROTECTIVE COMPOUNDS ACTIVATING NRF2 SIGNALING IN PRIMARY CULTURES OF RAT HEPATOCYTES

Nrf2 has emerged as a transcription factor playing a critical role in the metabolism and elimination of potentially harmful exogenous chemicals and their metabolites. By increasing the cellular power of detoxification, Nrf2 activators could potentially act as hepatoprotective agents against drug-induced liver injury.

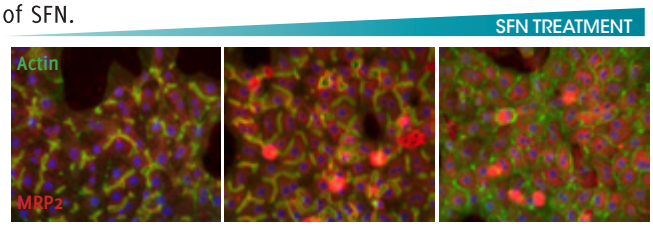
Using Sulforaphane (SFN), an organosulfur compound found in cruciferous vegetables (broccoli), which is known to activate the Nrf2 pathway, we determined the experimental conditions to monitor Nrf2 activation and identify drugs with hepatoprotective activity.

1 - HIGH-CONTENT ANALYSIS
SULFORAPHANE ACTIVATES THE NRF2 PATHWAY

Nrf2 regulates the expression of a number of genes involved in detoxification, including heme oxygenase 1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1), glutathione-S-transferase (GST), glutamate–cysteine ligase (Glc), cytochrome P450s (CYPs) and multidrug-resistant proteins (MRPs). We analyzed dose-dependent effects of SFN on the Nrf2 pathway by quantifying the expression level of Nrf2 targets.



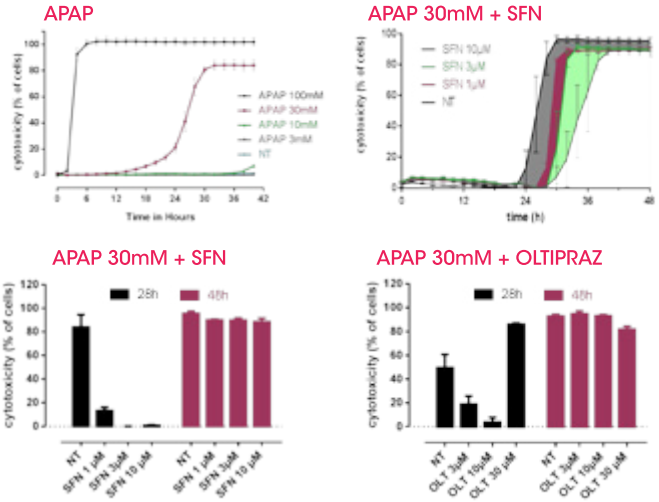
Among Nrf2 targets, MRP-2 is an efflux transporter that promotes biliary excretion and mediates the transport of xenobiotic substances, as well as GSH, out of the cell. The multiplexed quantification of MRP2 and actin in rat hepatocyte primary cultures treated with raising doses of SFN shows a dose-dependent increase of MRP2 in canaliculi, followed by MRP2 internalization and collapse of bile canaliculi network, corresponding to a toxic effect of SFN at higher doses. Quantification of GSH content also demonstrates a biphasic effect of SFN.



DOSE-DEPENDENT EFFECTS OF SULPHORAPHANE ON BILE CANALICULI STRUCTURE AND MRP2 EXPRESSION/LOCALIZATION

2 - LIVE CONTENT IMAGING
SULFORAPHANE DELAYS ACETAMINOPHEN-INDUCED HEPATOTOXICITY

Acetaminophen (APAP), better known as paracetamol, is a reference compound to assess drug-induced liver injury mediated by oxidative stress. Through a real-time cytotoxicity assay, we determined the optimal dose and time window to assess SFN hepatoprotective effects against APAP cytotoxicity.



Results show that SFN delays in a dose-dependent manner the hepatotoxic effects of APAP up to 12 hours. Note that a time point analysis could have provided very different results: for instance 3 µM SFN confers 100% of protection at 28h vs no protection at 48h. Similar results were obtained with an other known Nrf2 activator, Oltipraz.

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