Pharmacological Activation of Nrf2 Enhances Functional Liver Regeneration

Benjamin K.Y. Chan,1,2,18 Mohamed Elmasry,1,2 Shiva S. Forootan,1,4 Giusy Russomanno,1,4 Tobias M. Bunday,1 Fang Zhang,1 Nathalie Brabant,1 Philip J. Starkey Lewis,2,3 Rhona Aird,2 Emanuele Ricci,4 Timothy D. Andrews,3 Rowena L. Sison-Young,1 Amy L. Schofield,1 Yongxiang Fang,6 Adam Lister,1 Jack W. Sharkey,7 Harish Poptani,7 Neil R. Kitteringham,1 Stuart J. Forbes,1,3 Hassan Z. Malik,2 Stephen W. Fenwick,2 B. Kevin Park,1 Christopher E. Goldring,1,4 and Ian M. Copple1,18

BACKGROUND AND AIMS: The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) regulates an array of cytoprotective genes, yet studies in transgenic mice have led to conflicting reports on its role in liver regeneration. We aimed to test the hypothesis that pharmacological activation of Nrf2 would enhance liver regeneration.

APPROACH AND RESULTS: Wild-type and Nrf2 null mice were administered bardoxolone methyl (CDDO-Me), a potent activator of Nrf2 that has entered clinical development, and then subjected to two-thirds partial hepatectomy. Using translational noninvasive imaging techniques, CDDO-Me was shown to enhance the rate of restoration of liver volume (MRI) and improve liver function (multispectral optoacoustic imaging of indocyanine green clearance) in wild-type, but not Nrf2 null, mice following partial hepatectomy. Using immunofluorescence imaging and whole transcriptome analysis, these effects were found to be associated with an increase in hepatocyte hypertrophy and proliferation, the suppression of immune and inflammatory signals, and metabolic adaptation in the remnant liver tissue. Similar processes were modulated following exposure of primary human hepatocytes to CDDO-Me, highlighting the potential relevance of our findings to patients.

CONCLUSIONS: Our results indicate that pharmacological activation of Nrf2 is a promising strategy for enhancing functional liver regeneration. Such an approach could therefore aid the recovery of patients undergoing liver surgery and support the treatment of acute and chronic liver disease. (Hepatology 2021;74:973-986).

The regenerative capacity of the liver is well known, yet in many clinical scenarios inadequate liver regeneration can lead to liver impairment or failure.1 In the surgical setting, post-hepatectomy liver failure (PHLF) is a major complication with significant mortality following liver resection.2 Consequently, patients with a high risk of PHLF due to a small future liver remnant (FLR) and/or impaired parenchymal health are often deemed inoperable, preventing access to the only curative treatment for liver cancer. In addition, the poor regeneration of partial grafts from living donors can compromise the success of liver transplants. In the liver disease setting, inadequate regeneration can contribute to acute liver injury associated with drug toxicity and viral infection, and chronic parenchymal diseases characterized by marked fibrosis. As the burden of acute and chronic liver disease continues to rise,3 there is a need to identify
therapeutic strategies designed to promote liver regeneration and support hepatic function.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master transcriptional regulator of a battery of antioxidant, metabolic and cytoprotective genes, and has become a promising therapeutic target in several diseases with underlying oxidative stress and inflammatory components. However, the role of Nrf2 in liver regeneration is currently unclear, following conflicting reports that transgenic mice in which Nrf2 is either constitutively inactivated or activated exhibit impaired liver regeneration following two-thirds partial hepatectomy (PHx). With the burgeoning interest in the development and clinical use of small-molecule Nrf2 activators, it is now pertinent to examine the value of pharmacologically activating Nrf2 as a means of enhancing liver regeneration, yet no such investigations have been reported to date. Therefore, we have used translational noninvasive imaging techniques to assess the restoration of liver volume and functional integrity following PHx in mice treated with bardoxolone methyl (methyl-2-cyano 3,12-dioxooleano-1,9-dien-28-oate [CDDO-Me]), which is one of the most potent Nrf2 activators to have entered the clinic.

Our results indicate that CDDO-Me enhances the rate of restoration of liver volume and improves liver function in an Nrf2-dependent manner. These effects are associated with an increase in hepatocyte hypertrophy and proliferation, the suppression of immune and inflammatory signals, and metabolic adaptation in the remnant liver tissue. We also show that similar processes are modulated following exposure of primary human hepatocytes to CDDO-Me, highlighting pharmacological activation of Nrf2 as a promising strategy for enhancing functional liver regeneration.

**Experimental Procedures**

**ANIMALS**

All experiments were performed in accordance with a license granted under the UK Animals (Scientific Procedures) Act 1986 and were approved by the University of Liverpool Animal Ethics Committee. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals. Male C57BL/6J mice aged 8-10 weeks were supplied by Charles River (UK). All animals were housed in cages of ≤5 mice, in a...
temperature-controlled and humidity-controlled room, with 12-hour light/dark cycles. All animals received food and water ad libitum. Generation of Nrf2−/− mice and genotyping of progeny have been described previously. Mice were administered 3 mg/kg CDDO- Me (Cayman Chemical, Ann Arbor, MI) by intraperitoneal injection 24 hours and 1 hour before and 24 hours and 72 hours after two-thirds PHx (days −1, 0, 1 and 3, respectively). CDDO- Me was solubilized in 100% DMSO and administered at 1 μL/g. As DMSO did not affect liver regeneration following PHx (Supporting Fig. S1), untreated mice were used as controls to mimic the clinical setting.

**PRIMARY HEPATOCYTE ISOLATION AND CULTURE**

Human liver tissue was obtained by qualified medical staff at Aintree University Hospital (Liverpool, UK). All patients donated tissue as part of planned liver resections (Supporting Table S1). Written, informed consent was obtained from each patient. The study protocol was approved by the National Health Service North West–Liverpool Central Research Ethics Committee (11/NW/0327) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Hepatocytes were isolated from human liver as previously described. Cells were seeded at a density of 1 × 10^6 cells/mL onto Type I Collagen-coated plates (Beckton Dickinson, Franklin Lakes, NJ) and maintained at 37°C in a 5% CO2 atmosphere. After being allowed to adhere to the plates for a total of 16 hours, cells were exposed to 0.5% DMSO or 100 nM CDDO- Me for 24 hours.

**STATISTICAL ANALYSIS**

Statistical analysis was performed, as indicated in each figure legend, using Stats Direct 3 software. P values are reported to three decimal places. For Student t tests and Mann-Whitney U tests, two-tailed P values are reported. Differences were considered significant at P ≤ 0.05.

**SUPPLEMENTARY EXPERIMENTAL PROCEDURES**

See the Supporting Information for details on PHx, MRI, immunoblotting, quantitative PCR and TaqMan low-density array analysis, histology, cell-size determination, serum biomarker analyses, multispectral optoacoustic tomography (MSOT), RNA-sequencing and TempO-Seq high-throughput transcriptomics, Gene Set Enrichment Analysis (GSEA), and liver-tissue biochemical analyses.

**Results**

**PHARMACOLOGICAL ACTIVATION OF Nrf2 ENHANCES RESTORATION OF LIVER VOLUME FOLLOWING PHx**

To assess the potential therapeutic value of stimulating Nrf2 to enhance liver regeneration, we used MRI to measure liver volume before and after two-thirds PHx in mice treated with or without the Nrf2 activator CDDO- Me, which was administered before and after surgery to ensure maximal Nrf2 activation (Fig. 1A–C). Following PHx in control wild-type mice, liver volume increased in a time-dependent manner, returning to 84 ± 11% of baseline at day 7 (Fig. 1D). However, in wild-type mice treated with CDDO- Me, liver volume had already reached this level (83 ± 10% of baseline) by day 3 (Fig. 1D), indicating a significantly enhanced rate of liver regeneration. In control Nrf2−/− mice, liver regeneration was slightly, but not significantly, delayed compared with wild-type counterparts (Fig. 1D). However, the enhanced liver regeneration observed in CDDO- Me-treated wild-type mice was absent in CDDO- Me-treated Nrf2−/− mice (Fig. 1D), indicating the Nrf2-dependence of the enhanced restoration of liver volume observed following treatment with CDDO- Me. In a separate longer-term study, MRI revealed that liver volume plateaued around presurgery levels from day 14 in both control and CDDO- Me-treated wild-type mice (Fig. 1E), reflecting the efficient termination of liver regeneration, despite its early enhancement by CDDO- Me. Finally, nonlinear regression analysis of the relative liver volume data from the 31-day study (Supporting Fig. S2) confirmed that the restoration of liver volume was more than twice as rapid in mice treated with CDDO- Me compared with controls (Fig. 1F). Taken together, these data show that CDDO- Me markedly enhances liver regeneration following PHx in an Nrf2-dependent manner.
We next performed a complementary study in which mice were culled at a series of time points following PHx to enable collection of liver tissue and serum (Fig. 2A). Consistent with the MRI data, the restoration of liver-to-body weight ratio was enhanced in mice treated with CDDO-Me, from as early as 8 hours after PHx (Fig. 2B,C). To investigate whether the enhanced restoration of liver volume and mass observed in CDDO-Me-treated mice translated into improved parenchymal health, we first measured circulating markers of liver injury (alanine aminotransferase [ALT]) and function (total bilirubin [TBIL]). In control mice, PHx evoked significant early increases in ALT and TBIL, signifying a marked liver impairment (Fig. 2D,E). Importantly, CDDO-Me did not further increase the levels of ALT or TBIL (Fig. 2D,E). In fact, there was a trend toward improved levels of the markers in CDDO-Me-treated mice at early time points, although differences with control mice were not statistically significant (Fig. 2D,E). We next determined the clearance kinetics (half-life) of indocyanine green (ICG) from blood using MSOT imaging (Fig. 2F,G). ICG undergoes exclusive biliary excretion and is used in experimental and clinical settings to assess liver function.\(^{13,14}\) As we published

---

**FIG. 1.** Pharmacological activation of Nrf2 enhances restoration of liver volume following PHx. (A) Study design. Mice were treated with or without CDDO-Me, and PHx was performed on day 0. Liver volume was measured by MRI at the indicated times before and after PHx. (B) Overview of MRI volumetry method. The dashed yellow line defines the region of interest for volumetric analysis. (C) Representative coronal MRI sections of the livers of wild-type mice treated with or without CDDO-Me, imaged on the indicated days after PHx. (D) Liver volume in wild-type and Nrf2\(^{-/-}\) mice, expressed relative to the levels on day -1 (i.e., before PHx) (Kruskal-Wallis with Conover-Inman pairwise comparisons). Indicated \(P\) values are for the comparison of wild-type control and CDDO-Me-treated mice at each time point. All other comparisons yielded \(P\) values > 0.05. (E) Liver volume in wild-type mice imaged for 31 days following PHx. Unpaired Student \(t\) test, control versus CDDO-Me at each time point. (F) Time required to reach the indicated liver volumes, based on nonlinear regression analysis of the MRI data from (E). Unpaired Student \(t\) test, control versus CDDO-Me. Data represent the mean ± SD; \(n = 3-5\) per group. Abbreviations: KO, knockout; WT, wild-type.

---

**PHARMACOLOGICAL ACTIVATION OF Nrf2 SUPPORTS THE MAINTENANCE OF LIVER FUNCTION FOLLOWING PHx**
A

Time: d-1 d0 8h d1 d2 d3 d4 d5 d6 d7 d31
CDDO-Me: ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲
PHx: ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲
Tissue/serum: ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲

B

Control

CDDO-Me

8 h Day 2 Day 7

C

Liver Weight (% Body Weight)

MSOT:

Control

CDDO-Me

Liver Weight (% Body Weight)

D

ALT (U/L)

Time (post PHx)

E

TBIL (µmol/L)

Time (post PHx)

F

Time: d-1 d0 d1 d2 d3 d4 d5 d6 d7
CDDO-Me: ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲
PHx: ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲
MSOT: ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲

G

H

ICG Half-life (sec)

Nrf2 WT Control

Nrf2 WT CDDO-Me

Nrf2 KO Control

Nrf2 KO CDDO-ME
previously,\(^{(13)}\) PHx resulted in a significant increase in ICG half-life on days 1 and 2 in control mice, consistent with a loss of liver function, followed by a gradual recovery toward the baseline elimination rate by day 7 (Fig. 2H). Notably, ICG half-life was decreased by CDDO-Me in the days immediately following PHx (Fig. 2H), indicating an increase in the ability of the liver to eliminate ICG. This post-PHx preservation of liver function was not observed in CDDO-Me-treated Nrf2\(^{-/-}\) mice, in which ICG half-life essentially tracked the levels observed in wild-type control mice (Fig. 2H). Overall, these data indicate that pharmacological activation of Nrf2 supports the maintenance of liver function following PHx.

**PHARMACOLOGICAL ACTIVATION OF Nrf2 ENHANCES LIVER REGENERATION BY SUPPORTING HEPATOCYTOPLASMIC HYPERPLASIA**

The early phase of the regenerative response to PHx has been shown to be driven by compensatory hypertrophy of hepatocytes in the remnant liver tissue.\(^{(14)}\) Given that the remnant livers of CDDO-Me-treated mice were significantly larger than those of control mice as early as 8 hours following PHx (Figs. 1D,E and 2C), we hypothesized that pharmacological activation of Nrf2 enhanced liver regeneration by supporting the early hypertrophic response. We therefore performed immunofluorescent imaging of liver-tissue sections stained with phalloidin to enable membrane visualization and quantification of cell area. The early liver cell hypertrophy induced by PHx was found to be significantly enhanced by CDDO-Me (Fig. 3A,B). To verify that this effect was driven by hepatocytes, we co-stained cells with an antibody targeting hepatocyte nuclear factor 4 alpha (HNF4α) and used automated imaging analysis to quantify the area of HNF4α-positive cells (Supporting Fig. S3). This confirmed that CDDO-Me significantly enhanced the early hepatocyte hypertrophy induced by PHx (Fig. 3C). There was no significant difference between control and CDDO-Me-treated mice in the ratio of nuclei to cell area at any time point (Supporting Fig. S3), indicating that hepatocytes were not aberrantly enlarged. Hepatocellular hypertrophy is typically associated with the induction of enzymes involved in xenobiotic detoxication.\(^{(25)}\) Using custom gene-expression arrays, we found that a number of such enzymes were significantly up-regulated in the livers of CDDO-Me-treated mice 1 day after PHx (Fig. 3D). Finally, histopathological examination of the livers revealed that treatment with CDDO-Me was not associated with any adverse effects, with the livers of both groups of mice appearing normal by 31 days after PHx (Fig. 3E). Taken together, these data indicate that pharmacological activation of Nrf2 enhances liver regeneration by supporting an adaptive hepatocellular hypertrophy response.

**PHARMACOLOGICAL ACTIVATION OF Nrf2 ENHANCES LIVER REGENERATION BY ENHANCING HEPATOCYTOPLASMIC PROLIFERATION, SUPPRESSING IMMUNE/INFLAMMATORY SIGNALS, AND SUPPORTING METABOLIC ADAPTATION**

To examine the transcriptional responses that underlie the Nrf2-driven enhancement of liver...
A Day-1 8 h Day 2 Day 7 Day 31

Control  CDDO-Me

B Cell Area (µm²)

C Control  CDDO-Me

D mRNA Level (% Baseline)

E Day-1 8 h Day 2 Day 7 Day 31

Control  CDDO-Me
other tissues, (16) together with a more robust activation of genes involved in ribosome biogenesis, which is consistent with previous reports, (15) and highlighted the earlier and extended activation of these processes in the livers of mice treated with CDDO- Me (Fig. 4A). We also noted a stronger early up-regulation of the cell proliferation marker proliferating cell nuclear antigen (Pcna) and the Nrf2-regulated detoxication enzyme Nqo1 in the livers of CDDO- Me-treated mice (Fig. 4C,D). The enhanced activation of cell-proliferation processes in mice treated with CDDO- Me was consistent with a trend of extended mitosis in the remnant livers of these animals, compared with control mice, following PHx (Fig. 4B). We validated these responses at the protein level, by demonstrating the enhanced up-regulation of the cell proliferation marker proliferating cell nuclear antigen (Pcna) and the Nrf2-regulated detoxication enzyme Nqo1 in the livers of CDDO- Me-treated mice (Fig. 4C,D). The enhanced activation of cell-proliferation processes in mice treated with CDDO- Me was consistent with a trend of extended mitosis in the remnant livers of these animals, compared with control mice, following PHx (Supporting Fig. S6). Based on previous work showing a role for Nrf2 as a regulator of redox and metabolic processes, (17) and recent reports that remodeling of these processes contributes to liver regeneration, (18) we quantified hepatic glutathione levels and found that CDDO- Me prevented the loss of this important redox factor that was apparent in control mice following PHx (Fig. 5A). We also measured levels of nicotinamide adenine dinucleotide phosphate (NADP+) and its reduced form NADPH in liver tissue from control and CDDO- Me-treated mice following PHx (Fig. 5B,C). PHx itself provoked an increase in the level of both co-factors, and this was further enhanced...
inflammatory signals and supporting metabolic adaptation. Mice were treated with or without CDDO-Me, and PHx was performed on day 0. (A) Heatmaps showing GSEA normalized enrichment scores of selected gene ontology (GO) terms enriched with genes found to be differentially expressed at the indicated times after PHx, relative to d−1. Individual GO terms (q value ≤ 0.05) were clustered into parent terms, and selected parent/individual GO terms are displayed. See Supporting Table S3 for a full list of individual and parent GO terms. (B) mRNA expression levels of representative genes implicated in the indicated processes before and after PHx. Samples are pooled from 4−5 mice per time point. (C) Protein expression levels of Nqo1 and Pcna at the indicated times before and after PHx. (D) Densitometric analysis of immunoblots performed on nonpooled samples. Nqo1 and Pcna levels were normalized to β-actin. Mann-Whitney U test, control versus CDDO-Me at each time point. Data represent the mean ± SD; n = 4−5 per group. Abbreviations: Ccr9, chemokine (C-C motif) receptor 9; Gclm, glutamate-cysteine ligase modifier subunit; Il18, interleukin 18; MAPK, mitogen-activated protein kinase; Mcm2, minichromosome maintenance complex component 2; NES, normalized enrichment score; Nqo1, NAD(P)H dehydrogenase, quinone 1; Pcna, proliferating cell nuclear antigen; Pgd, phosphogluconate dehydrogenase; Scd1, stearoyl-Coenzyme A desaturase 1.

Taken together, these data suggest that pharmacological activation of Nrf2 enhances liver regeneration by promoting hepatocellular proliferation, suppressing immune/inflammatory signals and supporting metabolic adaptation (Fig. 5D).
Finally, to bridge our in vivo findings to humans, we exposed primary hepatocytes from human donors to CDDO-Me in vitro, and performed high-throughput transcriptomics analysis followed by GSEA. CDDO-Me did not alter the expression level of genes associated with cell proliferation (Fig. 6A), although this was not surprising given the resistance of primary hepatocytes to proliferate in vitro. However, consistent with our findings in mice, GSEA revealed that CDDO-Me caused the suppression of genes associated with immune and inflammatory signaling, the up-regulation of oxidative stress response genes, and metabolic adaptation in human hepatocytes (Fig. 6A). We used quantitative PCR to confirm the altered expression of representative genes from these pathways (Fig. 6B). Hence, these data show that several gene processes associated with enhanced liver regeneration in mice are similarly modulated following pharmacological activation of Nrf2 in primary human hepatocytes, highlighting the potential translational relevance of our findings.

Discussion

The increasing burden of liver disease now accounts for approximately 2 million deaths per year worldwide and a growing demand for liver transplantation. Furthermore, improvements in surgical techniques and neoadjuvant therapies have led to an increase in the number of patients undergoing surgery for resection of primary or secondary liver tumors, which carries a risk of PHLF. In each of these settings, strategies to boost the regenerative and functional capacity of the liver would provide a powerful clinical
FIG. 6. Pharmacological activation of Nrf2 in primary human hepatocytes modulates gene processes associated with enhanced liver regeneration in mice. Primary human hepatocytes were treated with CDDO-Me or vehicle control (DMSO) for 24 hours. (A) GSEA normalized enrichment scores and P values of selected GO terms significantly enriched with genes found to be differentially expressed in response to CDDO-Me. Individual GO terms (P < 0.05) were clustered into parent terms, which are displayed in the plot. See Supporting Table S4 for a full list of individual and parent GO terms. (B) mRNA expression levels of representative genes implicated in the indicated processes. Paired Student t test, control versus CDDO-Me at each time point. Data represent the mean ± SD; n = 5 donors. Abbreviations: CCL3, C- C motif chemokine ligand 3; ERK, extracellular signal-regulated kinase; GCLM, glutamate-cysteine ligase modifier subunit; IL18, interleukin 18; MAPK, mitogen-activated protein kinase; MCM2, minichromosome maintenance complex component 2; NAD, nicotinamide adenine dinucleotide; NES, normalized enrichment score; NQO1, NAD(P)H quinone dehydrogenase 1; PCNA, proliferating cell nuclear antigen; PGD, phosphogluconate dehydrogenase; SCD, stearoyl-CoA desaturase; STAT, signal transducer and activator of transcription.
tool. There is considerable interest in the potential of cell-based therapies to repopulate the impaired liver with healthy parenchyma and to support the repair of damaged cells. For example, macrophage therapy has recently been shown to be safe in patients with cirrhosis, with trials aiming to demonstrate efficacy ongoing. To complement cell-based approaches, there is a need for pharmacological strategies to enhance the regenerative process in patients with acute or chronic liver impairment, particularly where drugs already in clinical use can be repurposed. In this study, we have provided molecular, cellular, anatomical, and functional evidence that a small molecule Nrf2 activator that is undergoing clinical testing for the treatment of chronic kidney disease and several other pathologies can also improve liver regeneration. In supporting a pro-regenerative role for Nrf2 in the liver, our findings contrast with previous studies using transgenic mice that exhibit constitutive and strong activation of Nrf2, which does not reflect the pharmacological modulation that would be achieved in the clinical setting. Therefore, Nrf2 appears to be a promising therapeutic target for supporting normal liver function.

Following PHx in mice, liver regeneration is driven by an early, adaptive hepatocellular hypertrophy response, followed by the proliferation of a subset of hepatocytes that ultimately restores the liver to its original volume and functional capacity. Notably, our study demonstrates the role of Nrf2 in enhancing both of these aspects of liver regeneration following PHx. Although the contribution of hypertrophy to the regenerative process is less appreciated than that of cell proliferation, the former has been shown to be capable of enabling complete liver regeneration following PHx in pharmacologic and transgenic rodent models in which hepatocyte proliferation is impaired. Zou et al. previously reported the suppression of hepatocyte hypertrophy in the livers of Nrf2 knockout mice following PHx, while He et al. recently reported a hypertrophy-driven increase in liver-to-body weight ratio, in the absence of liver injury, in transgenic mice in which Nrf2 is constitutively activated within hepatocytes. In addition, the diminished liver regeneration in Nrf2 knockout mice following PHx has been associated with the inhibition of hepatocyte proliferation. Therefore, pharmacological activation of Nrf2 enhances two major components of the hepatic regenerative response.

We have also demonstrated that the enhanced liver regeneration observed in CDDO-Me-treated mice is associated with the suppression of immune cell activation and inflammatory signals. Dysregulation of these processes has been shown to inhibit liver regeneration and be associated with the progression of several liver pathologies, including fibrosis, cirrhosis, and hepatocellular carcinoma. Furthermore, our transcriptomic and biochemical analyses provide evidence for metabolic adaptation, accompanied by increases in the levels of NADP/H and glutathione, in the livers of mice treated with CDDO-Me. We and others have previously demonstrated a role for Nrf2 in the regulation of genes that coordinate redox balance, lipid metabolism, provision of cellular fuels, and other pathways that are vital to maintain homeostasis in the context of acute and chronic cellular insult. It is known that such processes are modulated in the immediate days following PHx, and it appears that such metabolic adaptations enable the generation of sufficient energy and biomass to maintain immediate hepatic function and drive the subsequent cell- and tissue-level responses underlying successful liver regeneration.

We have shown that pharmacological activation of Nrf2 in primary human hepatocytes results in the modulation of genes that are associated with enhanced liver regeneration in mice treated with CDDO-Me. In addition, it has recently been reported that many of these processes are also modulated in the remnant liver within 4 hours of surgery in patients. These findings highlight the potential translational importance of our work. Several small molecule Nrf2 activators are now either licensed for use in patients or undergoing clinical testing in a range of disease areas, whereas a number of pharmaceutical companies are actively developing the next generation of Nrf2 modulators. To develop further the pharmacological activation of Nrf2 as a strategy for enhancing liver regeneration, it will be important to test the effectiveness of these alternative compounds, and to confirm our findings in liver-disease models to ensure that Nrf2-mediated regeneration is conserved when the liver parenchyma is impaired. In this regard, it is encouraging to note that Sharma et al. recently demonstrated that an experimental Nrf2 activator reversed insulin resistance and suppressed hepatic steatosis and fibrosis in a mouse model of non-alcoholic...
Hepatology, Vol. 74, No. 2, 2021

In the surgical setting, portal vein embolization or ligation alone, or in combination with surgical splitting of the liver (known as associated liver partition and portal vein ligation for staged hepatectomy [ALPPS]), can be performed in selected patients to stimulate hypertrophy of the FLR before hepatectomy, and thus reduce the risk of PHLF. However, the use of ALPPS remains controversial due to the relatively high morbidity and mortality associated with the procedure. Shirasaki et al. (40) previously reported that an analogue of CDDO-Me enhanced the hypertrophy of nonligated liver lobes following portal vein branch ligation in mice. Taken together with our findings that pharmacological activation of Nrf2 enhances the immediate drivers (i.e., hepatocellular hypertrophy and proliferation) of liver regeneration following PHx, this raises the possibility of Nrf2 activators being used peri-operatively to increase the FLR and enhance liver regeneration in patients that would otherwise be deemed ineligible for curative surgery due to concern of PHLF. The advantage of this approach is that an Nrf2 activator can be administered over a short time frame close to the point of surgery, minimizing the risk of enhanced tumorigenicity associated with the constitutive hyperactivation of Nrf2 signaling that has been reported in tumors harboring somatic mutations in the Nrf2 gene or that of its inhibitor Kelch-like associated erythroid cell-derived protein with cap ‘n’ collar homology-associated protein 1 (Keap1) (41).

Nevertheless, it will be important to establish that even transient pharmacological activation of Nrf2 is not associated with an increase in residual tumor burden and/or greater risk of recurrence. This will provide a platform to test this strategy in suitable patient cohorts, thereby further establishing Nrf2 as a viable therapeutic target in the clinic.

Author Contributions: B.K.Y.C. contributed to the study concept, experiments, data interpretation, manuscript draft, and funding acquisition. M.E. contributed to the study concept, experiments, data interpretation, and manuscript draft. S.S.F., G.R., T.M.B., and P.J.S.L. contributed to the experiments, data interpretation, and manuscript draft. F.Z., N.B., E.R., T.D.A., and J.W.S. contributed to the experiments and data interpretation. R.A., R.L.S.Y., A.L.S., and Y.F. contributed to the experiments. A.L. contributed to the data interpretation.

H.P. contributed to the funding acquisition. N.R.K., H.Z.M., and S.W.F. contributed to the study concept and manuscript draft. S.J.F. contributed to the manuscript draft and funding acquisition. B.K.P. and C.E.G. contributed to the study concept, manuscript draft, and funding acquisition. I.M.C. contributed to the study concept, data interpretation, manuscript draft, and funding acquisition.

REFERENCES

1) Forbes SJ, Newsome PN. Liver regeneration–mechanisms and models to clinical application. Nat Rev Gastroenterol Hepatol 2016;13:473-485.
2) Bagante F, Ruzzenente A, Beal EW, Campagnaro T, Merath K, Conci S, et al. Complications after liver surgery: a benchmark analysis. HPB (Oxford) 2019;21:1139-1149.
3) Asrani SK, Devabhaktuni H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70:151-171.
4) Yamamoto M, Kessler TW, Motohashi H. The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 2018;98:1169-1203.
5) Cuadrado A, Rojo AI, Wells G, Hayes JD, Cousin SP, Ramsey WL, et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discov 2019;18:295-317.
6) Beyer TA, Xu W, Teupser D, auf dem Keller U, Bugnon P, Hildt E, et al. Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance. EMBO J 2008;27:212-223.
7) Wakabayashi N, Shin S, Slocum SL, Agoston ES, Wakabayashi J, Kwak M-K, et al. Regulation of notch1 signaling by nrf2: implications for tissue regeneration. Sci Signal 2010;3:ra52.
8) Kühler UA, Kurinna S, Schwitter D, Marti A, Schäfer M, Hellerbrand C, et al. Activated Nrf2 impairs liver regeneration in mice by activation of genes involved in cell-cycle control and apoptosis. Hepatology 2014;60:670-678.
9) Hu M, Zou Y, Nambari SM, Lee J, Yang Y, Dai G. Keap1 modulates the redox cycle and hepatocyte cell cycle in regenerating liver. Cell Cycle 2014;13:2349-2358.
10) Copple IM, Shelton LM, Walsh J, Kratschmar DV, Lister A, Odermatt A, et al. Chemical tuning enhances both potency toward nrf2 and in vitro therapeutic index of triperenoids. Toxicol Sci 2014;140:462-469.
11) Ioh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997;236:313-322.
12) Copple IM, den Hollander W, Callegaro G, Mutter FE, Maggs JL, Schofield AL, et al. Characterisation of the Nrf2 transcriptional network and its response to chemical insult in primary human hepatocytes: implications for prediction of drug-induced liver injury. Arch Toxicol 2019;93:385-399.
13) Brannin N, Elmasry M, Burton NC, Rodriguez JM, Starkey JW, Fenwick S, et al. Dynamic and accurate assessment of acetaminophen-induced hepatotoxicity by integrated pharmacokinetic imaging and mechanistic biomarkers in vivo. Toxicol Appl Pharmacol 2017;332:64-74.
14) Dousse D, Vibert E, Nicolas Q, Terasawa M, Cano L, Allard M-A, et al. Indocyanine green fluorescence imaging to predict graft survival after orthotopic liver transplantation: a pilot study. Liver Transpl 2020;26:1263-1274.
15) Michalopoulos GK. Hepatostat: liver regeneration and normal liver tissue maintenance. Hepatology 2017;65:1384-1392.
16) Sinturel F, Gerber A, Mauvoisin D, Wang J, Gatfield D, Stubblefield JJ, et al. Diurnal oscillations in liver mass and cell size accompany ribosome assembly cycles. Cell 2017;169:651-663.e614.
17) Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci 2014;39:199-218.
18) Caldez MJ, Van Hul N, Koh HWL, Teo XQ, Fan JJ, Tan PY, et al. Metabolic remodeling during liver regeneration. Dev Cell 2018;47:425-438.e425.
19) Passot G, Soubrane O, Giulianite F, Zimmitti G, Goere D, Yamashita S, et al. Recent advances in chemotherapy and surgery for colorectal liver metastases. Liver Cancer 2016;6:72-79.
20) Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: from liver transplantation to cell factory. J Hepatol 2015;62:S157-S169.
21) Moroni F, Dwyer BJ, Graham C, Pass C, Bailey L, Ritchie L, et al. Safety profile of autologous macrophage therapy for liver cirrhosis. Nat Med 2019;25:1560-1565.
22) Xu D, Xu M, Jeong S, Qian Y, Wu H, Xia Q, et al. The role of Nrf2 in liver disease: novel molecular mechanisms and therapeutic approaches. Front Pharmacol 2018;9:1428.
23) He L, Pu W, Liu X, Zhang Z, Han M, Li YI, et al. Proliferation tracing reveals regional hepatocyte generation in liver homeostasis and repair. Science 2021;371:eabc4346.
24) Diril MK, Ratnacaram CK, Padmakumar VC, Du T, Wasser M, Coppola V, et al. Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. Proc Natl Acad Sci U S A 2012;109:3826-3831.
25) Haga S, Ogawa W, Inoue H, Terui K, Ogino T, Igarashi R, et al. Compensatory recovery of liver mass by Akt-mediated hepatocellular hypertrophy in liver-specific STAT3-deficient mice. J Hepatol 2005;43:799-807.
26) Nagy P, Teramoto T, Factor VM, Sanchez A, Schnur J, Paku S, et al. Reconstitution of liver mass via cellular hypertrophy in the rat. Hepatology 2001;33:339-345.
27) Zou Y, Lee J, Nambari SM, Hu M, Rui W, Bao QI, et al. Nrf2 is involved in maintaining hepatocyte identity during liver regeneration. PLoS One 2014;9:e107423.
28) He F, Antonucci L, Yamachika S, Zhang Z, Taniguchi K, Umemura A, et al. NRF2 activates growth factor genes and downstream AKT signaling to induce mouse and human hepatomegaly. J Hepatol 2020;72:1182-1195.
29) Zou Y, Hu M, Lee J, Nambari SM, Garcia V, Bao QI, et al. Nrf2 is essential for timely M phase entry of replicating hepatocytes during liver regeneration. Am J Physiol Gastrointest Liver Physiol 2015;308:G262-G268.
30) Ishikawa M, Brooks AJ, Fernandez-Rojo MA, Medina J, Chhabra Y, Minami S, et al. Growth hormone stops excessive inflamma tion after partial hepatectomy, allowing liver regeneration and survival through induction of H2-BI/HLA-G. Hepatology 2021;73:759-775.
31) Robinson MW, Harmon C, O’Farrell C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol 2016;13:267-276.
32) Kitteringham NR, Abdullah A, Walsh J, Randle L, Jenkins RE, Sison R, et al. Proteomic analysis of Nrf2 deficient transgenic mice reveals cellular defence and lipid metabolism as primary Nrf2-dependent pathways in the liver. J Proteomics 2010;73:1612-1631.
33) Huang J, Rudnick DA. Elucidating the metabolic regulation of liver regeneration. Am J Pathol 2014;184:309-321.
34) Bhat M, Basini E, Baciu C, Angeli M, Humar A, Macparland S, et al. The basis of liver regeneration: a systems biology approach. Ann Hepatol 2019;18:422-428.
35) Lawrence MC, Darden CM, Vasu S, Kumanou K, Gu J, Wang X, et al. Profiling gene programs in the blood during liver regeneration in living liver donors. Liver Transpl 2019;25:1541-1560.
36) Sharma RS, Harrison DJ, Kiselevsky D, Cassidy DM, McNeilly AD, Gallagher JR, et al. Experimental nonalcoholic steatohepatitis and liver fibrosis are ameliorated by pharmacologic activation of Nrf2 (NF-E2 p45-related factor 2). Cell Mol Gastroenterol Hepatol 2018;5:367-398.
37) Mohs A, Otto T, Schneider KM, Peltzer M, Boekschoten M, Holland CH, et al. Hepatocyte-specific Nrf2 activation controls fibrogenesis and carcinogenesis in steatohepatitis. J Hepatol 2021;74:638-648.
38) Fragoulis A, Schenkel J, Herzog M, Schellenberg T, Jahr H, Pufe T, et al. Nrf2 ameliorates DDC-induced sclerosing cholangitis and biliary fibrosis and improves the regenerative capacity of the liver. Toxicol Sci 2019;169:485-498.
39) Schadde E, Arcales V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R, et al. Early survival and safety of ALPPS: first report of the International ALPPS Registry. Ann Surg 2014;260:829-836.
40) Shirasaki K, Taguchi K, Unno M, Motohashi H, Yamamoto M. NF-E2-related factor 2 promotes compensatory liver hyper trophy after portal vein branch ligation in mice. Hepatology 2014;59:2371-2382.
41) Raghunath A, Sundarraj K, Arfuso F, Sethi G, Perumal E. Dysregulation of Nrf2 in hepatocellular carcinoma: role in cancer progression and chemoresistance. Cancers (Basel) 2018;10:481.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.31859/supinfo.