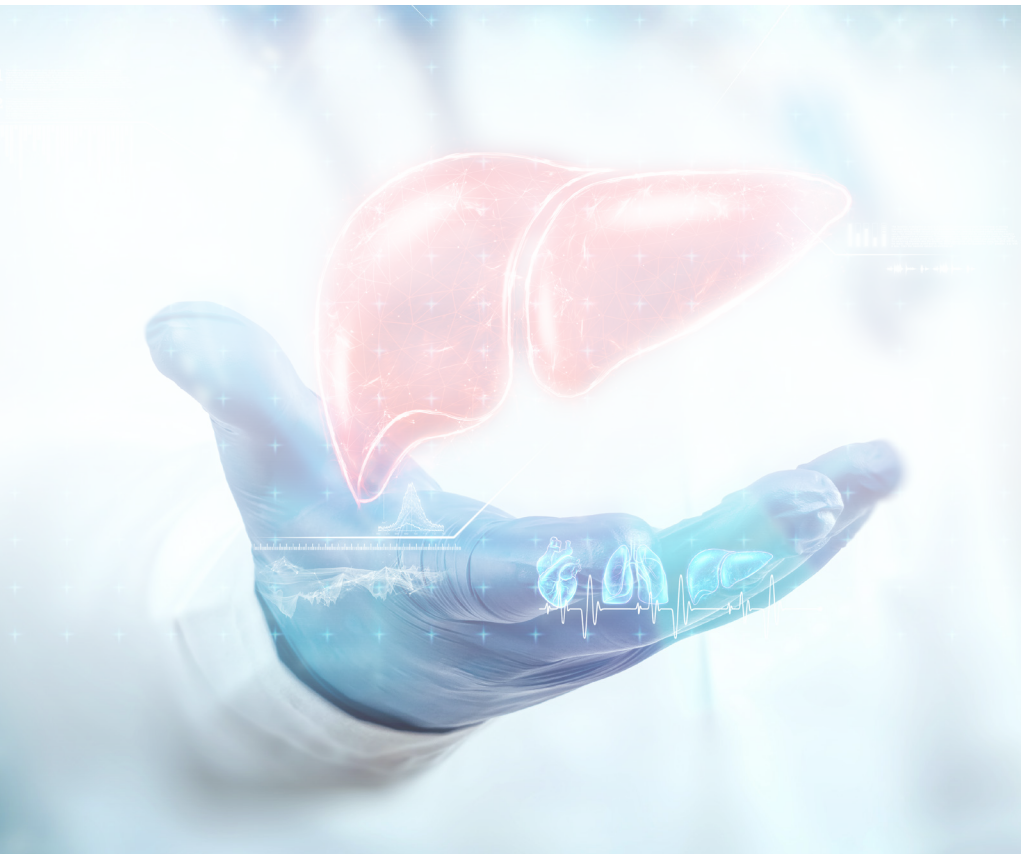

Challenge

Treatment for Non-Alcoholic Fatty Liver Disease

Solution

The InnuPure C16 automated nucleic acid extraction system provides a rapid and efficient method for extracting high-quality RNA for gene expression analysis of HK4, which can be used as a potential treatment for Non-Alcoholic Fatty Liver Diseases

Positive Allosteric GABAA Receptor Modulation Counteracts Lipotoxicity-Induced Gene Expression Changes in Hepatocytes In Vitro

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common liver disorder characterized by excessive fat accumulation in the liver. Lipotoxicity, a consequence of the collection of excess lipids, is one of the primary mechanisms contributing to NAFLD development. Positive allosteric modulators of GABAA receptors have been shown to have anti-inflammatory and anti-oxidative properties and may be a potential therapeutic target for the treatment of NAFLD. In this study, we investigated the effect of a positive allosteric modulator of GABAA receptors on lipotoxicity-induced gene expression changes in hepatocytes using the InnuPure C16 automated nucleic acid extraction system.

Abstract

Intending to treat non-alcoholic fatty liver disease, we focus on the positive allosteric modulator of the GABAA receptor, HK4, which has been seen to have hepatoprotective effects against lipotoxicity-induced apoptosis, ER stress, inflammation, and DNA damage in vitro.

In this experiment, HepG2 cells were treated and palmitate with and without HK4 for 4 hours; total RNA was extracted and assessed against the profiles of mRNAs through the DAVID database.

Through transcriptomic analysis, the following outcome came to fruition, some moderations were seen in genes as a response to palmitate lipotoxic stimulus, with 1457 genes causing effects on apoptosis, oxidative phosphorylation, ER stress, and mostly lipid metabolism.But the preincubation of HK4 restored the initial gene expression pattern of 456 genes by inhibiting the palmitate-induced dysregulation. Out of the 456 genes, 342 were upregulated and 113 downregulated.

The analysis of HK4 showed different pathways it targets. Through those pathways, it worked directly with transcription factors that deal with DNA repair, cell cycle, and ER Stress which prevent the lipotoxic mechanisms before the lipotoxic hepatocellular injury. Hence the conclusion suggests that HK4 can be a tremendous potential treatment for Non-Alcoholic fatty liver disease (NAFLD).

Thomas Scientific

analytikjena
An Endress+Hauser Company

Materials and Methods

Samples and reagents

- HepG2 cells (Darmstadt, Germany)
- Bovine serum albumin-conjugated palmitate (Cayman chemical, Ann Arbor, MI)
- HK4 (Taros Chemicals, Dortmund, Germany)
- RNA Isolation Kit (Analytik Jena, Jena, Germany)
- RNA High Sensitivity assay (Thermo Fisher Scientific Inc. Massachusetts, USA)
- Total RNA Standard Sensitivity Assay (Agilent Technologies Inc. Santa Clara, USA).
- QuantSeq 3′-mRNA-Seq Library Prep Kit FWD (Lexogen®, Vienna, Austria)

Instrumentation

- Innupure C16 (Analytik Jena,Jena, Germany)
- Qubit device (Thermo Fisher Scientific Inc. Massachusetts, USA)
- Capillary Electrophorèsis (Agilent Technologies Inc. Santa Clara, USA).
- NextSeq550 system (Illumina Inc. San Diego, USA)

Software

- Ingenuity Pathway Analysis (IPA, Qiagen)
- DAVID (Database for Annotation, Visualization, and Integrated Discovery)
- GraphPad Prism software (Version 8.0.1, San Diego, USA)
- CLC Genomics Workbench (version 22.0.1, QIAGEN, Venlo. NL)

The Workflow

There are two options in treating HepG2 cells: one being treated with bovine serum albumin-conjugated palmitate alone or with HK4 up to 30 minutes before exposure for 7 hours.
Innupure C16 using an RNA Isolation Kit, was able to extract the 5 total RNA comprising of (untreated, PA, PA + HK4) from HepG2.

InnuPure C16 touch combines highly precise liquid handling with automated extraction of high-quality nucleic acids. This instrument raises the bar when it comes to reliability and user-friendliness. The well-established walk-away principle ensures that the entire process is fully automated once the initial manual loading step is complete.
This feature consistently eliminates potential risks: dedicated ready-to-use reagent strips and/or plates make pipetting errors a thing of the past, while 1 mL pipette tips with aerosol filters effectively prevent contamination of the dispensing unit and samples. The (optional) UV lamp rules out additional contamination risks.
The integrated 10" tablet in combination with IPextract make the InnuPure C16 touch convenient to operate.

Figure 1: Innupure C16 touch

The following step was to measure the quality of the Total RNA samples that were 3′-mRNA seq and quantified by fluorometric measurement by Qubit device and RNA High sensitivity assay done by capillary electrophoresis.

The sample showed a RNA quality number (RQN 10) and satisfied library preparation using QuantSeq 3′-mRNA-Seq Library Prep Kit FWD; the bead-purified libraries were normalized and sequenced on the NextSeq550 system.

In sequencing, we observed alteration in protein-coding genes; 1457 genes were expressed compared to untreated and PA-treated hepatocytes, while half of 723 were expressed between PA alone and PA with HK4. On the lower side, 271 genes were expressed between untreated and PA with HK4, leading to upregulation and downregulation of genes.
The comparison can be seen when 784 untreated and 472 of PA+HK4 were upregulated compared to the PA, while 342 identic and 673 untreated were downgraded compared to the PA. Some of the genes, such as 342, were restored due to HK4, which derived from lipotoxicity. The full summary can be seen in Figure 2 below.

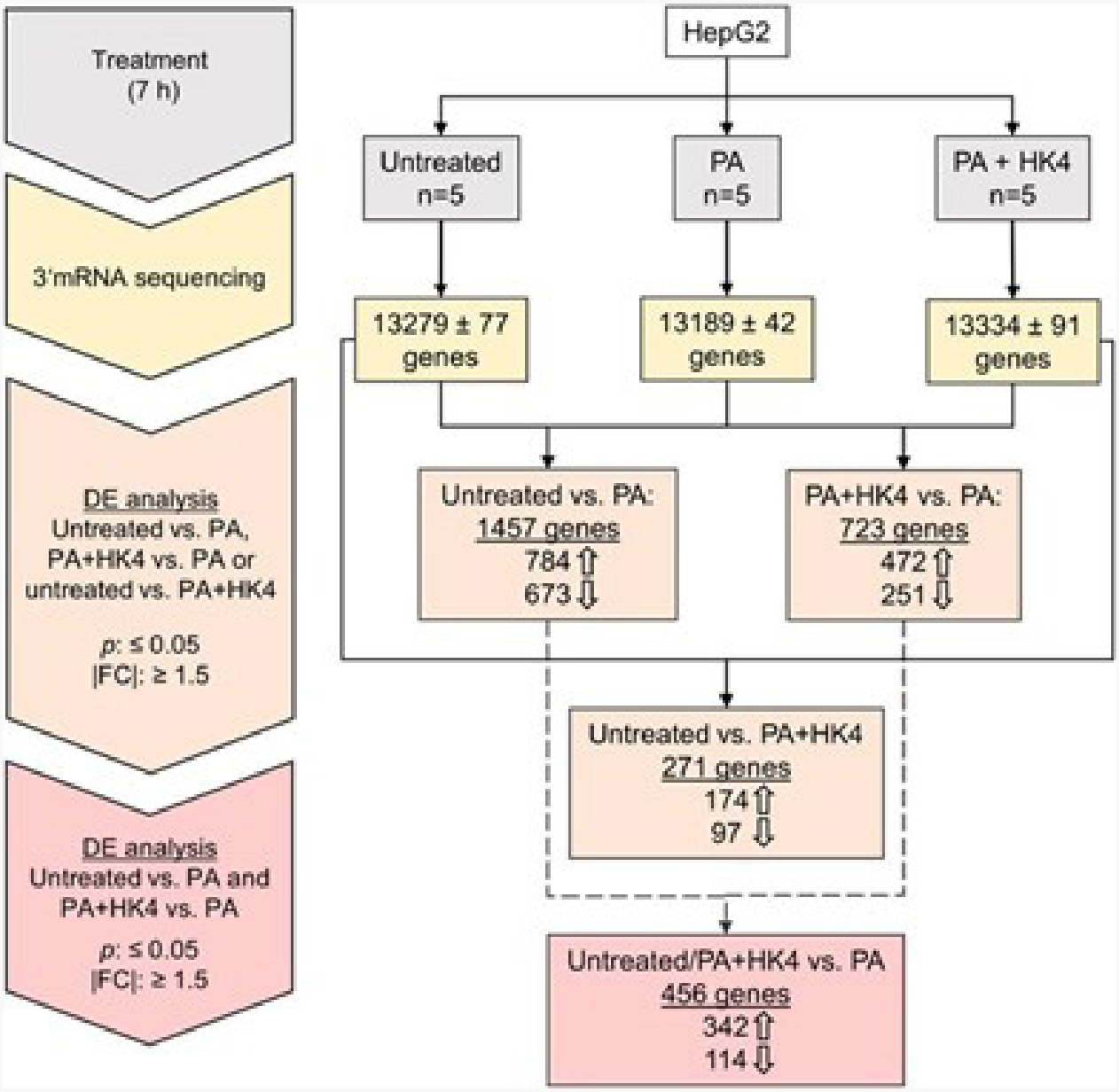

Figure 2: Workflow and overview of the number of genes in untreated, palmitate (PA) or PA + HK4-treated HepG2 cells. After 7 h treatment with 200 µM PA and 10 µM HK4, RNA has been isolated from five independent HepG2 cell lysates per treatment group and 3'-mRNA sequencing has been performed with Illumina NextSeq550 system. Thick arrows indicate up or downregulated genes in the first treatment group of the comparison. Differential expression (DE) of genes have been filtered by p-value ≤0.05 and an absolute value of fold change (|FC|) ≥ 1.5

Regarding how HK4 affects the gene expression pathway, some pathways were chosen to illustrate its role, including mitochondrial respiration, protein ubiquitination, apoptosis, and cell cycle.
The table below highlights the upregulation or downregulation of gene changes when treated with PA+HK4 compared to PA.

Table 1: Upregulation or downregulation of gene changes when treated with PA+HK4 compared to PA.

Pathway	Genes Up or Down regulated in PA+HK4 compared to PA
Mitochondrial respiration and Oxidative phosphorylation	14 genes were Upregulated 1 gene (ATP6V0A1) Downregulated
Protein ubiquitination	3 genes Downregulated (PELI3, ATG7 and DNAJC4)
Apoptosis	9 genes were Upregulated (TNFRSF10B, STEAP3, SIAH1, CTSC, BCL2L11, LMNB2, NRAS, DDIAS, DFFB) 5 genes were Downregulated (HRAS, BAX, TP73 and FASTK).
Cell Cycle	11 genes were Upregulated (CDCA7, CDC26 and CDC45), CDK7, CHEK2, MCM2 and ORC5 2 genes were Downregulated (CDKN1C, FZR1)

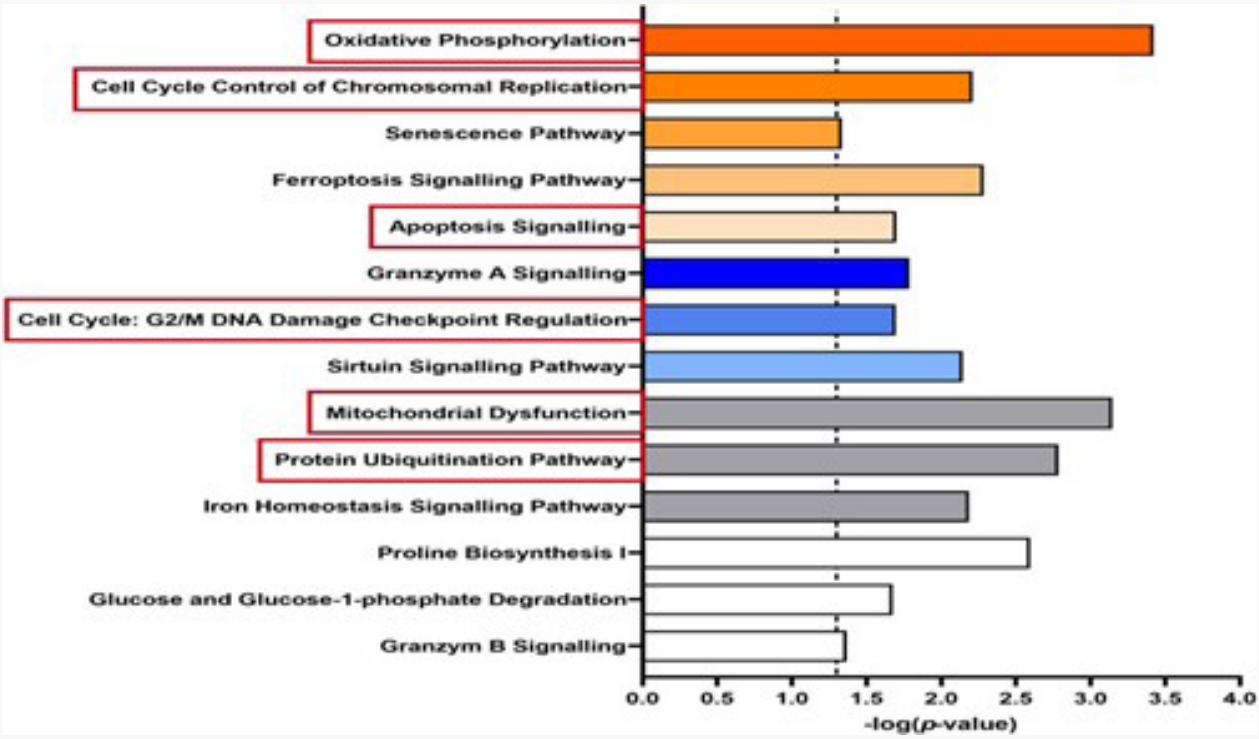

Figure 3: Enriched pathways of 456 differentially expressed genes in untreated or PA + HK4-treated HepG2 cells compared to PA-treated cells. The length of the bars is proportional to the significance of the association between the set of genes and the pathway, expressed by the negative logarithm of the p-value. Only pathways with $p \leq 0.05$ (dotted threshold line) are shown. Upregulated pathways in untreated or PA + HK4-treated cells associated with a positive z-score are colored in orange, downregulated pathway with a negative z-score is shown in blue and pathways with no change or an unknown pattern are marked in white or grey. Selected canonical pathways which are subsequently considered in more detail are highlighted in red.

Results and Discussion

The RNA analysis showed us changes in genes and cell functionality as HK4 preincubation resulted in the prevention of palmitate-induced dysregulation by restoring the initial gene expression pattern of untreated hepatocytes, which affects metabolic pathways such as lipid metabolism, oxidative phosphorylation, apoptosis, oxidative and ER stress.

Conclusion

Our findings suggest that positive allosteric modulation of GABAA receptors may have therapeutic potential for treating NAFLD through HK4 targeting and minimizing hepatocellular injury under a lipotoxic stimulus such as PA. The InnuPure C16 automated nucleic acid extraction system provided a rapid and efficient method for extracting high-quality RNA for gene expression analysis. This technology can study the mechanism of action of potential therapeutic targets for NAFLD and other liver disorders.

Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA902305.

References

[1] Bathish B., Robertson H., Dillon J. F., Dinkova-Kostova A. T., Hayes J. D. (2022). Nonalcoholic steatohepatitis and mechanisms by which it is ameliorated by activation of the CNC-bZIP transcription factor Nrf2. Free Radic. Biol. Med. 188, 221–261. doi:10.1016/j.freeradbiomed.2022.06.226
[2] Bisteau X., Caldez M. J., Kaldis P. (2014). The complex relationship between liver cancer and the cell cycle: A story of multiple regulations. Cancers 6 (1), 79–111. doi:10.3390/cancers6010079
[3] Brockhaus F., Brüne B. (1999). p53 accumulation in apoptotic macrophages is an energy demanding process that precedes cytochrome c release in response to nitric oxide. Oncogene 18 (47), 6403–6410. doi:10.1038/sj.onc.1203058
[4] Brykczynska U., Geigges M., Wiedemann S. J., Dror E., Böni-Schnetzler M., Hess C., et al. (2020). Distinct transcriptional responses across tissue-resident macrophages to short-term and long-term metabolic challenge. Cell. Rep. 30 (5), 1627–1643. doi:10.1016/j.celrep.2020.01.005
[5] Byrne C. D., Targher G. (2015). Nafld: A multisystem disease. J. hepatology 62 (1), S47–S64. doi:10.1016/j.jhep.2014.12.012
[6] Caldez M. J., Bjorklund M., Kaldis P. (2020). Cell cycle regulation in NAFLD: When imbalanced metabolism limits cell division. Hepatol. Int. 14 (4), 463–474. doi:10.1007/s12072-020-10066-6
[7] Da Huang W., Sherman B. T., Lempicki R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4 (1), 44–57. doi:10.1038/nprot.2008.211
[8] Dabravolski S. A., Bezsonov E. E., Orekhov A. N. (2021). The role of mitochondria dysfunction and hepatic senescence in NAFLD development and progression. Biomed. Pharmacother. = Biomedecine Pharmacother. 142, 112041. doi:10.1016/j.biopha.2021.112041
[9] Das S. K., Mondal A. K., Elbein S. C. (2010). Distinct gene expression profiles characterize cellular responses to palmitate and oleate. J. lipid Res. 51 (8), 2121–2131. doi:10.1194/jlr.M004275
[10] Dewidar B., Kahl S., Pafili K., Roden M. (2020). Metabolic liver disease in diabetes - from mechanisms to clinical trials. Metabolism 111S, 154299. clinical and experimental. doi:10.1016/j.metabol.2020.154299
[11] Dhanasekaran D. N., Reddy E. P. (2008). JNK signaling in apoptosis. Oncogene 27 (48), 6245–6251. doi:10.1038/onc.2008.301
[12] Engeland K. (2022). Cell cycle regulation: p53-p21-RB signaling. Cell. death Differ. 29 (5), 946–960. doi:10.1038/s41418-022-00988-z
[13] Fornari F., Gramantieri L., Ferracin M., Veronese A., Sabbioni S., Calin G. A., et al. (2008). MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. Oncogene 27 (43), 5651–5661. doi:10.1038/onc.2008.178
[14] Frazzi R. (2021). BIRC3 and BIRC5: Multi-faceted inhibitors in cancer. Cell. & Biosci. 11 (1), 8. doi:10.1186/s13578-020-00521-0
[15] French S., Bardag-Gorce F. (2005). "Ubiquitin-proteasome pathway in the pathogenesis of liver disease," in Signaling pathways in liver diseases: With 15 tables. Editor J.-F. Dufour (Berlin, Heidelberg: Springer), 377–389.
[16] Fromenty B., Roden M. (2022). Mitochondrial alterations in fatty liver diseases. J. hepatology 78, 415–429. doi:10.1016/j.jhep.2022.09.020
[17] Fu P.-Y., Hu B., Ma X.-L., Yang Z.-F., Yu M.-C., Sun H.-X., et al. (2019). New insight into BIRC3: A novel prognostic indicator and a potential therapeutic target for liver cancer. J. Cell. Biochem. 120 (4), 6035–6045. doi:10.1002/jcb.27890
[18] Greco D., Kotronen A., Westerbacka J., Puig O., Arkkila P., Kiviluoto T., et al. (2008). Gene expression in human NAFLD. Am. J. physiology. Gastrointest. liver physiology 294 (5), G1281–G1287. doi:10.1152/ajpgi.00074.2008

[19] Gross B., Pawlak M., Lefebvre P., Staels B. (2017). PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. Nat. Rev. Endocrinol. 13 (1), 36–49. doi:10.1038/nrendo.2016.135
[20] Hata T., Rehman F., Hori T., Nguyen J. H. (2019). GABA, γ-aminobutyric acid, protects against severe liver injury. J. Surg. Res. 236, 172–183. doi:10.1016/j.jss.2018.11.047
[21] He G., Karin M. (2011). NF-κB and STAT3 - key players in liver inflammation and cancer. Cell. Res. 21 (1), 159–168. doi:10.1038/cr.2010.183
[22] Hernández E. Á., Kahl S., Seelig A., Begovatz P., Irmler M., Kupriyanova Y., et al. (2017). Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. J. Clin. investigation 127 (2), 695–708. doi:10.1172/JCI89444
[23] Humpton T. J., Hall H., Kiourtis C., Nixon C., Clark W., Hedley A., et al. (2022). p53-mediated redox control promotes liver regeneration and maintains liver function in response to CCl4. Cell. death Differ. 29 (3), 514–526. doi:10.1038/s41418-021-00871-3
[24] Jelenik T., Kaul K., Séquaris G., Flögel U., Phielix E., Kotzka J., et al. (2017). Mechanisms of insulin resistance in primary and secondary nonalcoholic fatty liver. Diabetes 66 (8), 2241–2253. doi:10.2337/db16-1147
[25] Ji J., Zhang L., Wang P., Mu Y.-M., Zhu X.-Y., Wu Y.-Y., et al. (2005). Saturated free fatty acid, palmitic acid, induces apoptosis in fetal hepatocytes in culture. Exp. Toxicol. pathology official J. Gesellschaft fur Toxikologische Pathologie 56 (6), 369–376. doi:10.1016/j.etp.2005.02.003
[26] Ji F., Zhou M., Sun Z., Jiang Z., Zhu H., Xie Z., et al. (2021). Integrative proteomics reveals the role of E3 ubiquitin ligase SYVN1 in hepatocellular carcinoma metastasis. Cancer Commun. Lond. Engl. 41 (10), 1007–1023. doi:10.1002/cac2.12192
[27] Johnson R. F., Witzel I.-I., Perkins N. D. (2011). p53-dependent regulation of mitochondrial energy production by the RelA subunit of NF-κB. Cancer Res. 71 (16), 5588–5597. doi:10.1158/0008-5472.CAN-10-4252
[28] Jonas W., Schürmann A. (2021). Genetic and epigenetic factors determining NAFLD risk. Mol. Metab. 50, 101111. doi:10.1016/j.molmet.2020.101111
[29] Joshi-Barve S., Barve S. S., Butt W., Klein J., McClain C. J. (2003). Inhibition of proteasome function leads to NF-kappaB-independent IL-8 expression in human hepatocytes. Hepatol. Baltim. Md 38 (5), 1178–1187. doi:10.1053/jhep.2003.50470
[30] Kikkert M., Doolman R., Dai M., Avner R., Hassink G., van Voorden S., et al. (2004). Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. J. Biol. Chem. 279 (5), 3525–3534. doi:10.1074/jbc.M307453200
[31] Klein B. J., Piao L., Xi Y., Rincon-Arano H., Rothbart S. B., Peng D., et al. (2014). The histone-H3K4-specific demethylase KDM5B binds to its substrate and product through distinct PHD fingers. Cell. Rep. 6 (2), 325–335. doi:10.1016/j.celrep.2013.12.021
[32] Koliaki C., Roden M. (2016). Alterations of mitochondrial function and insulin sensitivity in human obesity and diabetes mellitus. Annu. Rev. Nutr. 36, 337–367. doi:10.1146/annurev-nutr-071715-050656
[33] Koliaki C., Szendroedi J., Kaul K., Jelenik T., Nowotny P., Jankowiak F., et al. (2015). Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell. metab. 21 (5), 739–746. doi:10.1016/j.cmet.2015.04.004
[34] Krämer A., Green J., Pollard J., Tugendreich S. (2014). Causal analysis approaches in ingenuity pathway analysis. Bioinforma. Oxf. Engl. 30 (4), 523–530. doi:10.1093/bioinformatics/btt703
[35] Kunst C., Haderer M., Heckel S., Schlosser S., Müller M. (2016). The p53 family in hepatocellular carcinoma. Transl. Cancer Res. 5 (6), 632–638. doi:10.21037/tcr.2016.11.79
[36] Lee I. H., Kawai Y., Fergusson M. M., Rovira I. I., Bishop A. J. R., Motoyama N., et al. (2012). Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. Sci. (New York, N.Y.) 336 (6078), 225–228. doi:10.1126/science.1218395
[37] Lee K., Haddad A., Osme A., Kim C., Borzou A., Ilchenko S., et al. (2018). Hepatic mitochondrial defects in a nonalcoholic fatty liver disease mouse model are associated with increased degradation of oxidative phosphorylation subunits. Mol. Cell. proteomics MCP 17 (12), 2371–2386. doi:10.1074/mcp.RA118.000961
[38] Li X., Liu L., Yang S., Song N., Zhou X., Gao J., et al. (2014). Histone demethylase KDM5B is a key regulator of genome stability. Proc. Natl. Acad. Sci. U. S. A. 111 (19), 7096–7101. doi:10.1073/pnas.1324036111
[39] Li W., Wong C. C., Zhang X., Kang W., Nakatsu G., Zhao Q., et al. (2018). CAB39L elicited an anti-Warburg effect via a LKB1-AMPK-PGC1α axis to inhibit gastric tumorigenesis. Oncogene 37 (50), 6383–6398. doi:10.1038/s41388-018-0402-1
[40] Li H., Lan T., Xu L., Liu H., Wang J., Li J., et al. (2020). NCSTN promotes hepatocellular carcinoma cell growth and metastasis via β-catenin activation in a Notch1/AKT dependent manner. J. Exp. Clin. cancer Res. CR 39 (1), 128. doi:10.1186/s13046-020-01638-3
[41] Li K., Zhang K., Wang H., Wu Y., Chen N., Chen J., et al. (2021). Hrd1-mediated ACLY ubiquitination alleviate NAFLD in db/db mice. Metabolism Clin. Exp. 114, 154349. doi:10.1016/j.metabol.2020.154349
[42] Li Y., Lin Y., Han X., Li W., Yan W., Ma Y., et al. (2021). GSK3 inhibitor ameliorates steatosis through the modulation of mitochondrial dysfunction in hepatocytes of obese patients. iScience 24 (3), 102149. doi:10.1016/j.isci.2021.102149
[43] Marcel V., Perrier S., Aoubala M., Ageorges S., Groves M. J., Diot A., et al. (2010). Δ160p53 is a novel N-terminal p53 isoform encoded by Δ133p53 transcript. FEBS Lett. 584 (21), 4463–4468. doi:10.1016/j.febslet.2010.10.005
[44] Minuk G. Y., Bear C. E., Sarjeant E. J. (1987). Sodium-independent, bicuculline-sensitive 3HGABA binding to isolated rat hepatocytes. Am. J. physiology 252 (5), G642–G647. doi:10.1152/ajpgi.1987.252.5.G642
[45] Mota M., Banini B. A., Cazanave S. C., Sanyal A. J. (2016). Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism Clin. Exp. 65 (8), 1049–1061. doi:10.1016/j.metabol.2016.02.014
[46] Murru E., Manca C., Carta G., Banni S. (2022). Impact of dietary palmitic acid on lipid metabolism. Front. Nutr. 9, 861664. doi:10.3389/fnut.2022.861664
[47] Nicol S. M., Bray S. E., Black H. D., Lorimore S. A., Wright E. G., Lane D. P., et al. (2013). The RNA helicase p68 (DDX5) is selectively required for the induction of p53-dependent p21 expression and cell-cycle arrest after DNA damage. Oncogene 32 (29), 3461–3469. doi:10.1038/onc.2012.426
[48] Norikura T., Kojima-Yuasa A., Opare Kennedy D., Matsui-Yuasa I. (2007). Protective effect of gamma-aminobutyric acid (GABA) against cytotoxicity of ethanol in isolated rat hepatocytes involves modulations in cellular polyamine levels. Amino acids 32 (3), 419–423. doi:10.1007/s00726-006-0381-3
[49] Nowotny B., Zahiragic L., Krog D., Nowotny P. J., Herder C., Carstensen M., et al. (2013). Mechanisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. Diabetes 62 (7), 2240–2248. doi:10.2337/db12-1179
[50] Panasiuk A., Dzieciol J., Panasiuk B., Prokopowicz D. (2006). Expression of p53, Bax and Bcl-2 proteins in hepatocytes in non-alcoholic fatty liver disease. World J. gastroenterology 12 (38), 6198–6202. doi:10.3748/wjg.v12.i38.6198
[51] Pérez-Schindler J., Vargas-Fernández E., Karrer-Cardel B., Ritz D., Schmidt A., Handschin C. (2022). Characterization of regulatory transcriptional mechanisms in hepatocyte lipotoxicity. Sci. Rep. 12 (1), 11477. doi:10.1038/s41598-022-15731-4
[52] Piccolis M., Bond L. M., Kampmann M., Pulimeno P., Chitraju C., Jayson C. B. K., et al. (2019). Probing the global cellular responses to lipotoxicity caused by saturated fatty acids. Mol. Cell. 74 (1), 32–44. doi:10.1016/j.molcel.2019.01.036
[53] Qu J., Zou T., Lin Z. (2021). The roles of the ubiquitin-proteasome system in the endoplasmic reticulum stress pathway. Int. J. Mol. Sci. 22 (4), 1526. doi:10.3390/ijms22041526
[54] Rodriguez-Lebron E., Denovan-Wright E. M., Nash K., Lewin A. S., Mandel R. J. (2005). Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. Mol. Ther. J. Am. Soc. Gene Ther. 12 (4), 618–633. doi:10.1016/j.ymthe.2005.05.006
[55] Rohbeck E., Hasse B., Koopmans G., Romero A., Belgardt B.-F., Roden M., et al. (2022). Positive allosteric γ-aminobutyric acid type A receptor modulation prevents lipotoxicity-induced injury in hepatocytes in vitro. Diabetes, Obes. metabolism 24 (8), 1498–1508. doi:10.1111/dom.14719
[55] Rutkowski D. T., Wu J., Back S.-H., Callaghan M. U., Ferris S. P., Iqbal J., et al. (2008). UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. Dev. Cell. 15 (6), 829–840. doi:10.1016/j.devcel.2008.10.015
[57] Ryaboshapkina M., Hammar M. (2017). Human hepatic gene expression signature of non-alcoholic fatty liver disease progression, a meta-analysis. Sci. Rep. 7 (1), 12361. doi:10.1038/s41598-017-10930-w

[58] Sharma L. K., Lu J., Bai Y. (2009). Mitochondrial respiratory complex I: Structure, function and implication in human diseases. Curr. Med. Chem. 16 (10), 1266–1277. doi:10.2174/092986709787846578
[59] Sherman B. T., Hao M., Qiu J., Jiao X., Baseler M. W., Lane H. C., et al. (2022). David: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic acids Res. 50, W216–W221. doi:10.1093/nar/gkac194
[60] Shilpa J., Roshni B. T., Chinthu R., Paulose C. S. (2012). Role of GABA and serotonin coupled chitosan nanoparticles in enhanced hepatocyte proliferation. J. Mater. Sci. Mater. Med. 23 (12), 2913–2921. doi:10.1007/s10856-012-4754-8
[61] Staňková P., Kučera O., Peterová E., Elkalaf M., Rychtrmoc D., Melek J., et al. (2021). Western diet decreases the liver mitochondrial oxidative flux of succinate: Insight from a murine NAFLD model. Int. J. Mol. Sci. 22, 6908. doi:10.3390/ijms22136908
[62] Steensels S., Qiao J., Ersoy B. A. (2020). Transcriptional regulation in non-alcoholic fatty liver disease. Metabolites 10, 283. doi:10.3390/metabo10070283
[63] Targher G., Corey K. E., Byrne C. D., Roden M. (2021). The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments. Nat. Rev. Gastroenterology hepatology 18 (9), 599–612. doi:10.1038/s41575-021-00448-y
[64] Tilg H., Moschen A. R., Roden M. (2017). NAFLD and diabetes mellitus. Nat. Rev. Gastroenterology hepatology 14 (1), 32–42. doi:10.1038/nrgastro.2016.147
[65] Valera A. G., Díaz-Hernández M., Hernández F., Ortega Z., Lucas J. J. (2005). The ubiquitin-proteasome system in Huntington's disease. Neurosci. a Rev. J. bringing Neurobiol. neurology psychiatry 11 (6), 583–594. doi:10.1177/1073858405280639
[66] Váraljai R., Islam A. B. M. M. K., Beshiri M. L., Rehman J., Lopez-Bigas N., Benevolenskaya E. V. (2015). Increased mitochondrial function downstream from KDM5A histone demethylase rescues differentiation in pRB-deficient cells. Genes. & Dev. 29 (17), 1817–1834. doi:10.1101/gad.264036.115
[67] Vesting A. J., Jais A., Klemm P., Steuernagel L., Wienand P., Fog-Tonnesen M., et al. (2022). NIK/MAP3K14 in hepatocytes orchestrates NASH to hepatocellular carcinoma progression via JAK2/STAT5 inhibition. Mol. Metab. 66, 101626. doi:10.1016/j.molmet.2022.101626
[68] Vock C., Gleissner M., Klapper M., Döring F. (2007). Identification of palmitate-regulated genes in HepG2 cells by applying microarray analysis. Biochimica biophysica acta 1770 (9), 1283–1288. doi:10.1016/j.bbagen.2007.07.001
[69] Vogelstein B., Lane D., Levine A. J. (2000). Surfing the p53 network. Nature 408 (6810), 307–310. doi:10.1038/35042675
[70] Wadgaonkar R., Phelps K. M., Haque Z., Williams A. J., Silverman E. S., Collins T. (1999). CREB-binding protein is a nuclear integrator of nuclear factor-kappaB and p53 signaling. J. Biol. Chem. 274 (4), 1879–1882. doi:10.1074/jbc.274.4.1879
[71] Wei J., Yuan Y., Chen L., Xu Y., Zhang Y., Wang Y., et al. (2018). ER-associated ubiquitin ligase HRD1 programs liver metabolism by targeting multiple metabolic enzymes. Nat. Commun. 9 (1), 3659. doi:10.1038/s41467-018-06091-7
[72] Wingelhofer B., Neubauer H. A., Valent P., Han X., Constantinescu S. N., Gunning P. T., et al. (2018). Implications of STAT3 and STAT5 signaling on gene regulation and chromatin remodeling in hematopoietic cancer. Leukemia 32 (8), 1713–1726. doi:10.1038/s41375-018-0117-x Wójcik C. (2002). Regulation of apoptosis by the ubiquitin and proteasome pathway. J. Cell. Mol. Med. 6 (1), 25–48. doi:10.1111/j.1582-4934.2002.tb00309.x
[73] Wu T., Zhao F., Gao B., Tan C., Yagishita N., Nakajima T., et al. (2014). Hrd1 suppresses Nrf2-mediated cellular protection during liver cirrhosis. Genes. & Dev. 28 (7), 708–722. doi:10.1101/gad.238246.114
[74] Xing Z., Russon M. P., Utturkar S. M., Tran E. J. (2020). The RNA helicase DDX5 supports mitochondrial function in small cell lung cancer. J. Biol. Chem. 295 (27), 8988–8998. doi:10.1074/jbc.RA120.012600
[75] Xu K., Sun S., Yan M., Cui J., Yang Y., Li W., et al. (2022). DDX5 and DDX17-multifaceted proteins in the regulation of tumorigenesis and tumor progression. Front. Oncol. 12, 943032. doi:10.3389/fonc.2022.943032
[76] BYamasaki S., Yagishita N., Sasaki T., Nakazawa M., Kato Y., Yamadera T., et al. (2007). Cytoplasmic destruction of p53 by the endoplasmic reticulum-resident ubiquitin ligase ‚Synoviolin. EMBO J. 26 (1), 113–122. doi:10.1038/sj.emboj.7601490
[77] Yan Z., Miao X., Zhang B., Xie J. (2018). p53 as a double-edged sword in the progression of non-alcoholic fatty liver disease. Life Sci. 215, 64–72. doi:10.1016/j.lfs.2018.10.051
[78] Yang H., Zhong X., Ballar P., Luo S., Shen Y., Rubinsztein D. C., et al. (2007). Ubiquitin ligase Hrd1 enhances the degradation and suppresses the toxicity of polyglutamine-expanded huntingtin. Exp. Cell. Res. 313 (3), 538–550. doi:10.1016/j.yexcr.2006.10.031
[79] Zhang Y., Ye S., Lu W., Zhong J., Leng Y., Yang T., et al. (2022). RNA helicase DEAD-box protein 5 alleviates nonalcoholic steatohepatitis progression via tethering TSC complex and suppressing mTORC1 signaling. Hepatol. Online ahead of print. doi:10.1002/hep.32651
[80] Zhou Z., He M., Shah A. A., Wan Y. (2016). Insights into APC/C: From cellular function to diseases and therapeutics. Cell. Div. 11, 9. doi:10.1186/s13008-016-0021-6