

SHORT COMMUNICATION

Depletion of three combined THOC5 mRNA export protein target genes synergistically induces human hepatocellular carcinoma cell death

S Saran^{1,4}, DDH Tran^{1,4}, F Ewald², A Koch¹, A Hoffmann³, M Koch², B Nashan² and T Tamura¹

Hepatocellular carcinoma (HCC) is a frequent form of cancer with a poor prognosis and with limited possibilities of medical intervention. It has been shown that over 100 putative driver genes are associated with multiple recurrently altered pathways in HCC, suggesting that multiple pathways will need to be inhibited for any therapeutic method. mRNA processing is regulated by a complex RNA–protein network that is essential for the maintenance of homeostasis. THOC5, a member of mRNA export complex, has a role in less than 1% of mRNA processing, and is required for cell growth and differentiation, but not for cell survival in normal fibroblasts, hepatocytes and macrophages. In this report, we show that 50% depletion of THOC5 in human HCC cell lines Huh7 and HepG2 induced apoptosis. Transcriptome analysis using THOC5-depleted cells revealed that 396 genes, such as transmembrane BAX inhibitor motif containing 4 (TMBIM4), transmembrane emp24-like trafficking protein 10 (Tmed10) and D-tyrosyl-tRNA deacylase 2 (Dtd2) genes were downregulated in both cell lines. The depletion of one of these THOC5 target genes in Huh7 or HepG2 did not significantly induce cell death, suggesting that these may be fine tuners for HCC cell survival. However, the depletion of a combination of these genes synergistically increased the number of TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling)-positive HCC. It must be noted that the depletion of these genes did not induce cell death in the hepatocyte cell line, THLE-2 cells. THOC5 expression was enhanced in 78% of cytological differentiation grading G2 and G3 tumor in primary HCC. Furthermore, the expression of a putative glycoprotein, Tmed10, is correlated to THOC5 expression level in primary HCCs, suggesting that this protein may be a novel biomarker for HCC. These data imply that the suppression of the multiple THOC5 target genes may represent a novel strategy for HCC therapy.

Oncogene (2016) 35, 3872–3879; doi:10.1038/onc.2015.433; published online 9 November 2015

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent tumor types world-wide,¹ however, current treatment options are limited and there are no precise and effective medical strategies.² Furthermore, biopsies for HCC diagnosis and biomarkers³ are of limited use. Risk factors for HCC such as viral infection, alcoholic liver injury or steatohepatitis lead to chronic hepatitis or cirrhosis (preneoplastic stages),² however, the molecular mechanisms by which HCC further develops are largely unknown. It has been recently shown by exome sequencing of HCCs that 161 putative driver genes are associated with 11 recurrently altered pathways in HCC development.⁴ These facts suggest that multiple target molecules will be required for effective HCC therapy.

The THO complex, which is a sub-complex of TREX (transcription/export), is conserved in species extending from *Drosophila melanogaster* to humans.^{5–7} The THO complex has a role in mRNA 3' processing, nuclear RNA export and genome stability.^{8–11} We have previously shown that THOC5 (or FMIP), a member of THO complex, knockout mice died before 5 days in the embryonic stage.¹² Furthermore, using inducible THOC5 knockout mice, we showed that THOC5 is required for the maintenance of hematopoietic and intestinal stem cells, however, mice lacking THOC5 in adult liver survived over 2 months,¹² suggesting that

THOC5 is required for the maintenance of primitive cells, but not required for the maintenance of terminally differentiated cells such as hepatocytes. In fibroblasts or bone marrow macrophages, less than 1% of mRNA processing was impaired upon the depletion of THOC5, and cells stopped proliferating, but did not undergo apoptosis.^{13,14} THOC5 is a nuclear/cytoplasm shuttling protein and substrate for several tyrosine kinases such as macrophage colony stimulating factor receptor, Fms¹⁵ and various leukemogenic tyrosine kinases, such as Bcr-Abl or NPM-ALK.¹⁶ Furthermore, THOC5 tyrosine phosphorylation is elevated in stem cells from patients with chronic myeloid leukemia,¹⁷ suggesting that THOC5 may be involved in leukemia development. In this report, we examined whether THOC5 is involved in HCC survival. Here, we show that upon depletion of THOC5 in HCC cell lines, Huh7 and HepG2 underwent cell death. Using this system, we identified several THOC5 target genes. The depletion of a set of THOC5 target genes induced death synergistically in HCC cells, but not in the human hepatocyte cell line, THLE-2. Furthermore, in primary HCCs, the expression level of THOC5 and its target molecules are elevated. These data suggest that THOC5 and its target molecules may be novel biomarkers for HCC and the suppression of multiple THOC5 target genes may offer new opportunities for HCC therapy.

¹Institut fuer Biochemie, OE4310, Medizinische Hochschule Hannover, Hannover, Germany; ²Department of Hepatobiliary and Transplant Surgery, University Medical Center Eppendorf, Hamburg, Germany and ³Klinik für Orthopädie, OE8893, Medizinische Hochschule Hannover, Hannover, Germany. Correspondence: Professor T Tamura, Institut fuer Biochemie, OE4310, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, Hannover D-30623, Germany.

E-mail: Tamura.Teruko@MH-Hannover.de

⁴These authors contributed equally to this work.

Received 23 July 2015; revised 1 October 2015; accepted 11 October 2015; published online 9 November 2015

RESULTS AND DISCUSSION

THOC5 depletion in human HCC cell lines, Huh7 and HepG2, induces cell death

To examine the role of THOC5 in HCC, we depleted THOC5 in two human HCC cell lines, Huh7 and HepG2, and immortalized human hepatocyte cell line THLE-2 using lentivirus carrying green fluorescent

protein (GFP) and a non-targeting control sequence or shTHOC5 (Lenti-GFP-shCr and Lenti-GFP-shTHOC5). Four days after infection with Lenti-GFP-shTHOC5, the THOC5 expression level was reduced by more than 50% in all cell lines (Figures 1a and b). Simultaneously, growth rate was reduced within 4 days after Lenti-GFP-shTHOC5 infection (Figure 1c). Notably, the level of THOC5 expression in the

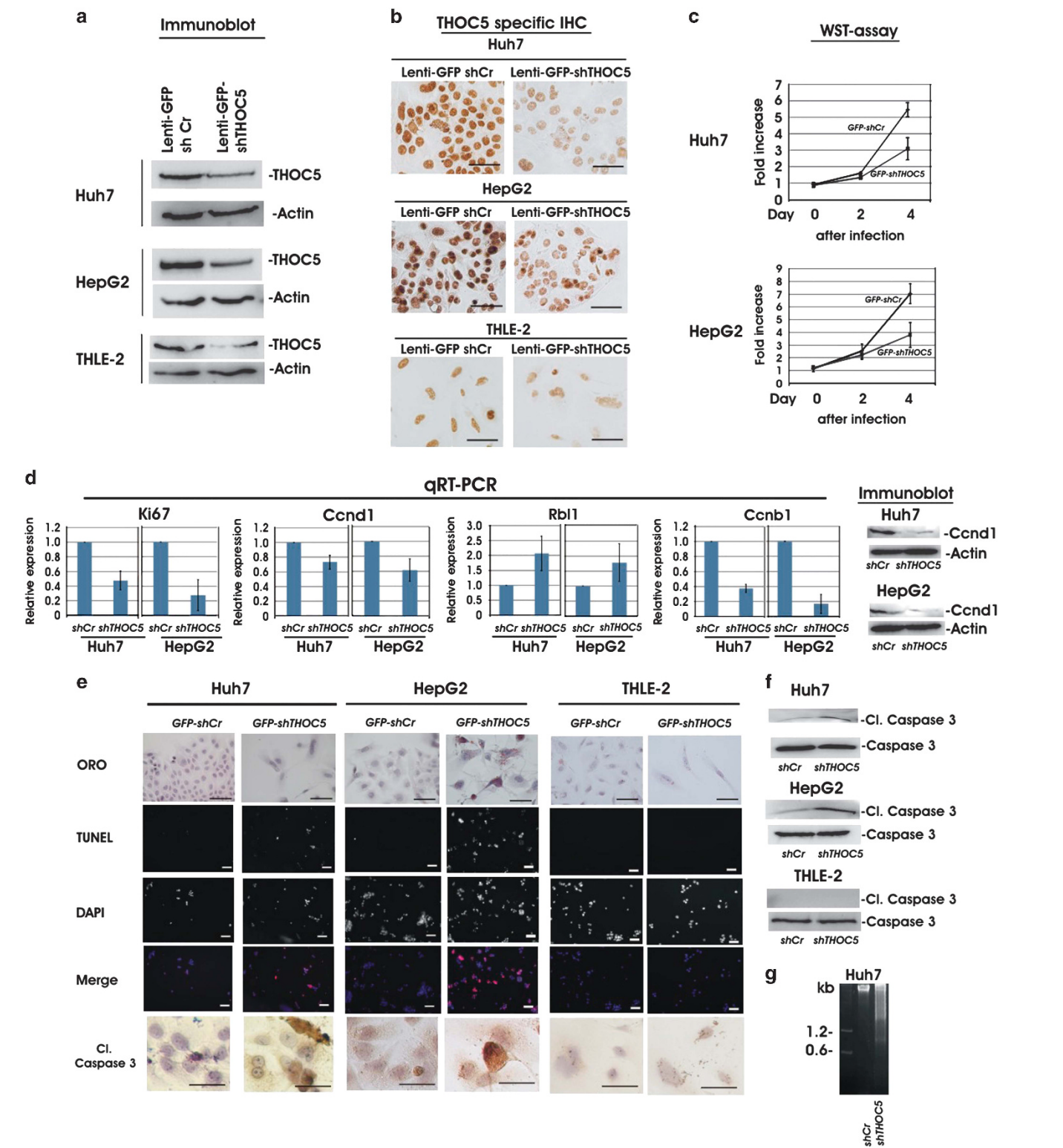


Figure 1. For caption see page 3874.

normal hepatocyte cell line, THLE-2, was approximately half of that in HCC cell lines, Huh7 or HepG2 (Figure 1b) and cells grew threefold to fivefold slower than both HCC cell lines. Upon depletion of THOC5 in HCC cell lines, cell cycle marker Ki67 and cyclin B1 (Ccnb1) expression were reduced by more than 50%, whereas G1 phase cell cycle blocker, retinoblastoma-like 1 (Rb1), expression was drastically increased (Figure 1d). Furthermore, cyclin D1 (Ccn1) mRNA and protein levels were also reduced (Figure 1d, RT-PCR and immunoblot), suggesting that the cell cycle G1-S progression was impaired upon depletion of THOC5. Five days after infection, TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) and cleaved caspase 3-positive cells appeared in Huh7 and HepG2 (Figures 1e and f), indicating that cells underwent apoptosis. In addition, DNA fragmentation (DNA ladder) was observed in Huh7 cells (Figure 1g). These data imply that the depletion of THOC5 in Huh7 and HepG2 cells impaired G1-S cell cycle progression and in parallel induced apoptosis. On the other hand, the depletion of THOC5 in THLE-2 cells did not cause cell death (Figures 1e and f). In addition, lipid droplet accumulation was observed in THOC5-depleted HepG2 cells (Figure 1e, Oil red O).

These data also confirm *in vivo* data showing that THOC5 is not required for the maintenance of normal liver cells,¹² but imply that it is required for the survival of HCC. To identify THOC5 target genes that have a role in the survival of HCC, we performed transcriptome analysis using THOC5-depleted Huh7 and HepG2 cell systems.

Identification of THOC5-dependent genes in human HCC cell lines Huh7 and HepG2 were infected with Lenti-GFP-shCr or Lenti-GFP-shTHOC5 and RNA was isolated from both cell lines 4 days after infection and supplied for transcriptome analysis. A total of 1248 and 836 genes were downregulated more than twofold upon the depletion of THOC5 in Huh7 and HepG2 cells, respectively (Figure 2a). Among the downregulated genes, 396 genes were common in these two cell lines (Figure 2a, Supplementary Table 1). Altogether 396 common downregulated genes were further analyzed on the cBioportal site: HCC (TCGA, provisional) (data from 373 HCC patient samples).^{18,19} We excluded 112 genes that show a positive correlation between survival and mRNA expression level (Pearson's correlation: > 0.04) from the list of 396 genes. We then further selected 105 genes that are expressed at a high level in untreated Huh7 and HepG2 cells (hybridization intensity greater than 2000, GEO series: GSE70178) (Figure 2a). These 105 genes are divided into three groups analyzed by Ingenuity Pathway Analysis (version 23814503) (Qiagen, Hilden, Germany), 23 genes that are known to be involved in HCC (I), 65 genes that are known to be involved in other types of cancer (II) and 17 genes that have not been shown in cancer involvement (III) (Figure 2a). To identify novel genes that are involved in cancer, we selected genes from the

category III. As one of the genes in this group is a pseudogene, we examined the remaining 16 genes. We first validated all 16 genes (Supplementary Table 2: *) by qRT-PCR. Among those 16 genes, we confirmed 7 genes by qRT-PCR (more than twofold reduction in both Huh7 and HepG2 upon depletion of THOC5), namely C11orf58, cytochrome c oxidase assembly factor (Cox20), coproporphyrinogen oxidase (Cpx), D-tyrosyl-tRNA deacylase 2 (Dtd2, putative), methyltransferase like 21A (Mettl21A), transmembrane and coiled-coil domain family 1 (Tmcc1) and transmembrane emp24-like trafficking protein 10 (Tmed10) (Figure 2b). In addition, we selected genes that may be involved in an anti-apoptosis signal from category II. In this category, only one gene, transmembrane BAX inhibitor motif containing 4 (TMIM4)²⁰ gene (Figure 2b), was suggested to be involved in an anti-apoptotic signal. To identify novel HCC-specific target genes, we excluded genes that are essential to maintain non-hepatic human kidney epithelial HK2 cells. Upon depletion of Cpx, C11orf58, Mettl21A, or Tmcc1, more than 50% of HK2 cells became TUNEL-positive within 3 days (Figure 2c), whereas depletion of Cox20, Dtd2, TMIM4 and Tmed10 in the same cells did not cause cell death (< 5% TUNEL-positive cells: Figure 2c*), suggesting that these four genes may be potential HCC-specific target genes.

Depletion of a set of THOC5 target genes, TMIM4, Tmed10 and Dtd2, synergistically induces cell death of human HCC cells

To examine whether these four genes are expressed in an HCC-specific manner, we compared the expression level of Cox20, Dtd2, TMIM4 and Tmed10 in Huh7, HepG2 and THLE-2 cells. Strikingly, Dtd2 was expressed in both HCC cell lines 5- to 10-fold higher than in THLE-2 cells (Figure 3a).

We next transfected each of the siRNAs against THOC5 (FMIP), and THOC5 target genes, Cox20, Dtd2, TMIM4, Tmed10, or control siRNA into Huh7, or HepG2 cells. To validate siRNA transfection, we examined the expression level of corresponding RNA after transfection by qRT-PCR. In all cases, the level was reduced to 50–80% (Figure 3b). Then, cells were stained with DAPI and TUNEL. In agreement with the data obtained from Lenti-GFP-shTHOC5 infection, siTHOC5 resulted in approximately 50% of all cells being TUNEL-positive within 3 days (Figures 3c and d), while the depletion of TMIM4 and Cox20 induced cell death in less than 7% of cells, and treatment with siTmed10 or siDtd2 induced cell death in 14–23% of Huh7 and HepG2 (Figures 3c and d).

These data suggest that Cox20, Dtd2, TMIM4 and Tmed10 may not be essential, but are fine tuners for HCC cell survival. We next depleted a combination of these genes in the same cells. Although the depletion of TMIM4, an apoptosis inhibitor,²¹ alone induced cell death in less than 3% of Huh7 cells and 7% of HepG2 cells, the

Figure 1. Knockdown of THOC5 in Huh7 and HepG2, but not THLE-2, cells caused apoptosis. HCC cell lines Huh7 and HepG2 were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum. THLE-2 cells (ATCC, Manassas, VA, USA; CRL-2706) were grown following the instructions provided. shRNAs against THOC5 and a non-silencing shRNA in pGIPZshRNAmir (scrambled shRNA control: RHS4346) were obtained from OpenBiosystems (Thermo Scientific, Fremont, CA, USA). Two shRNAs against THOC5 V2_27183 (ATCAAAGTACTGATTG) and V3_352773 (TGTATAACTCATAGTCTCT) were utilized. Lentivirus was generated from both shRNAs against THOC5 and from the scrambled shRNA control using a second generation packaging system. Cells were infected with Lentivirus carrying GFP and scrambled control shRNA (Lenti-GFP-shCr), or GFP and shTHOC5 (Lenti-GFP-shTHOC5). (a, b) Four days after infection, proteins were extracted and supplied for THOC5²⁵ and beta actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (Actin, loading control)-specific immunoblot. Details of immunoblotting have been described previously²⁶ (a), or cells were fixed and then supplied for THOC5-specific immunohistochemical (IHC) staining as described previously²⁶ (b). (c, d) Cells were infected with viruses, and 2 and 4 days after infection, proliferation rate was measured using a WST-1 proliferation assay kit (Roche Diagnostics, Mannheim, Germany). Numbers are mean ± s.d. (e), or RNA was isolated from sister culture (4 days) in (c) and Ki67-, Ccn1-, Rb1- and Ccnb1-specific qRT-PCR or protein was extracted from sister culture Ccn1 or actin (both from Santa Cruz Biotechnology) specific immunoblot were performed (d). (e–g) Cells were infected with viruses for 5 days and then were stained by oil red O (ORO), TUNEL (*in situ* cell death detection kit, Roche Diagnostics), IHC using cleaved caspase 3-specific antibody (Cell signaling technology, Cambridge, UK) or DAPI: 4',6-Diamidino-2-phenylindole. Bars represent 50 µm: three independent experiments were performed and an example of representative data is shown here (e), or sister culture from (e) were applied for cleavage caspase 3-specific immunoblot (f) or DNAs were separated on 1.5% agarose gel stained with ethidium bromide and photographed under UV light. M: base pair markers (g).

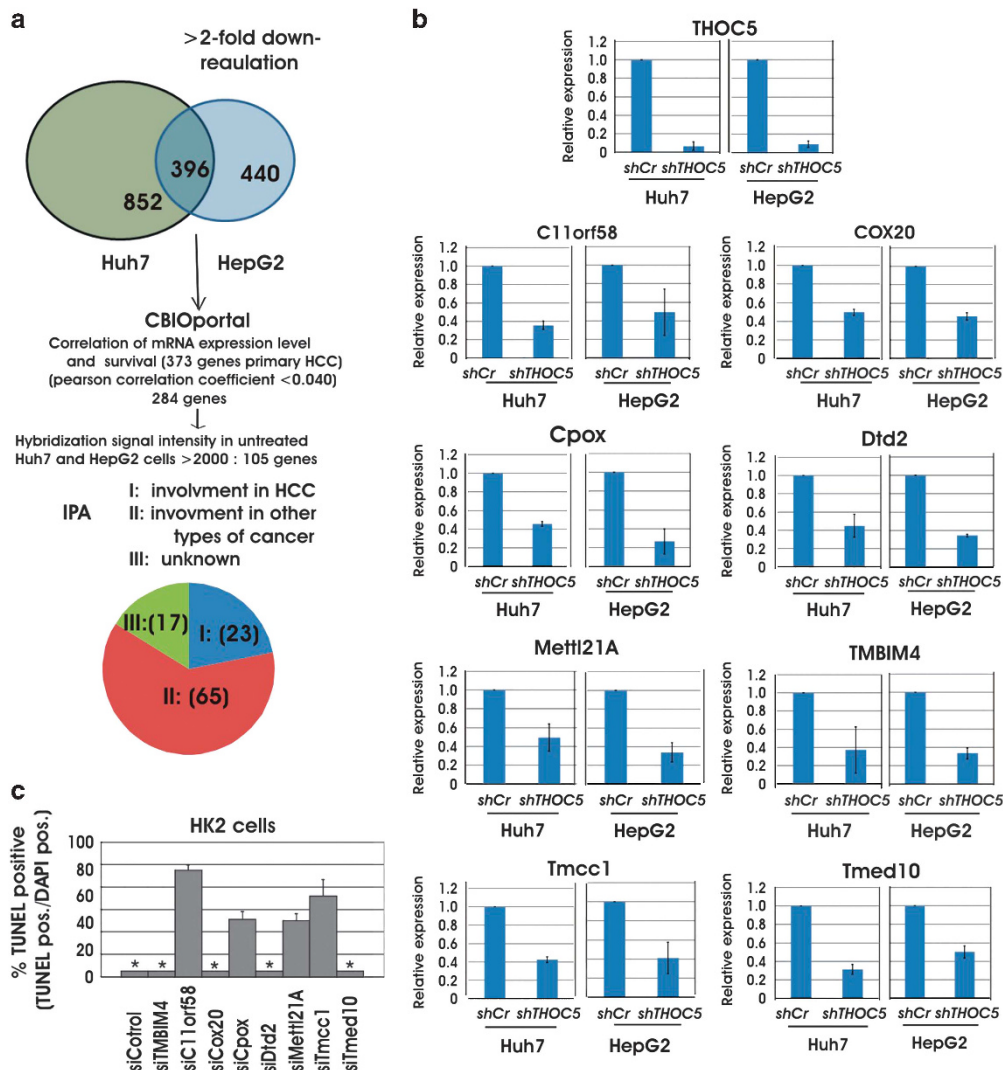


Figure 2. Downregulation of genes upon knockdown of THOC5 in Huh7 and HepG2. RNAs were isolated from Huh7 and HepG2 cells infected with Lenti-GFP-shCr or Lenti-GFP-shTHOC5 for 4 days, and supplied for transcriptome analysis. Dual-color Microarray experiments based on the Agilent (Santa Clara, CA, USA) platform have been performed as described previously.¹⁴ The complete microarray data along with processing protocols have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO series accession number GSE70178. **(a)** Venn diagram of total and common downregulated genes (> twofold) upon knockdown of THOC5 in Huh7 and HepG2. Common downregulated genes were analyzed by cBioportal site: HCC (TCGA, provisional), and by Ingenuity Pathway Analysis (IPA: version 23814503) (Qiagen). **(b)** Validation of transcriptome analysis data by qRT-PCR. RNA was isolated from cells with the High Pure RNA Isolation kit (Roche Diagnostics) according to the manufacturer's instructions. One microgram of RNA was reverse-transcribed using oligo dT primers and the Omniscript reverse transcriptase kit (Qiagen) following the instructions provided. One-twentieth of the cDNA mix was used for real-time PCR using 10 pmol of forward and reverse primer and SensiFAST SYBR No-ROX kit (Bioline, London, UK). THOC5-, C11orf58-, Cox20-, Cpox-, Dtd2-, Mettl21A-, TMBIM4-, Tmcc1-, and Tmed10-specific qRT-PCR in Huh7 and HepG2 cells infected with Lenti-GFP-shCr (shCr) and Lenti-GFP-shTHOC5 (shTHOC5). Primer pairs for each PCR are described in Supplementary Table 2. Numbers are mean \pm s.d. Three independent experiments were performed. **(c)** Control siRNA (sc-37007, Santa Cruz Biotechnology) and siRNAs against THOC5 target genes, TMBIM4 (GAAP, sc-96014), C11orf58 (sc-96887), Cox20 (sc-88765), Cpox (sc-77907), Dtd2 (sc-92275), Mettl21A (sc-94929), Tmcc1 (sc-77984) or Tmed10 (TMDP21, sc-63136) (total 25 pmol of siRNA for each transfection) were transfected into human kidney epithelial cells, HK2 (ATCC CRL-2190) cells, and 3 days after transfection, TUNEL assay was performed. Bars represent % of TUNEL-positive cells/200 DAPI-positive cells. Numbers are mean \pm s.d. Three independent experiments were performed. * < 5%.

combination of siTMBIM4 with siDtd2 (44% (Huh7) and 37% (HepG2) TUNEL-positive cells) and with siTmed10 (24% (Huh7) and 47% (HepG2) TUNEL-positive cells) but not with Cox20 (< 3%), synergistically enhanced the number of TUNEL-positive cells (Figure 3d). These data imply that when the cell death signal is triggered by knockdown of Dtd2 or Tmed10, the additional depletion of an apoptosis inhibitor, TMBIM4, drastically increased the cell death rate. In addition, the transfection with all three siRNAs in combination slightly increased cell death rate even further (Figure 3d).

It must be noted that depletion of the combination of these three genes in THLE-2 cells did not induce apoptosis (< 3% TUNEL-positive cells) (Figure 3d *).

As the Dtd2 gene was expressed at a higher level in Huh7 and HepG2 cells than in THLE-2 cells (Figure 3a), Dtd2 together with TMBIM4 may be promising candidates as specific target molecules for HCC therapy, however, the exact molecular function of Dtd2 remains to be studied. To examine the molecular function and the role of Dtd2 in HCC formation, we are currently generating antibodies against human Dtd2. These

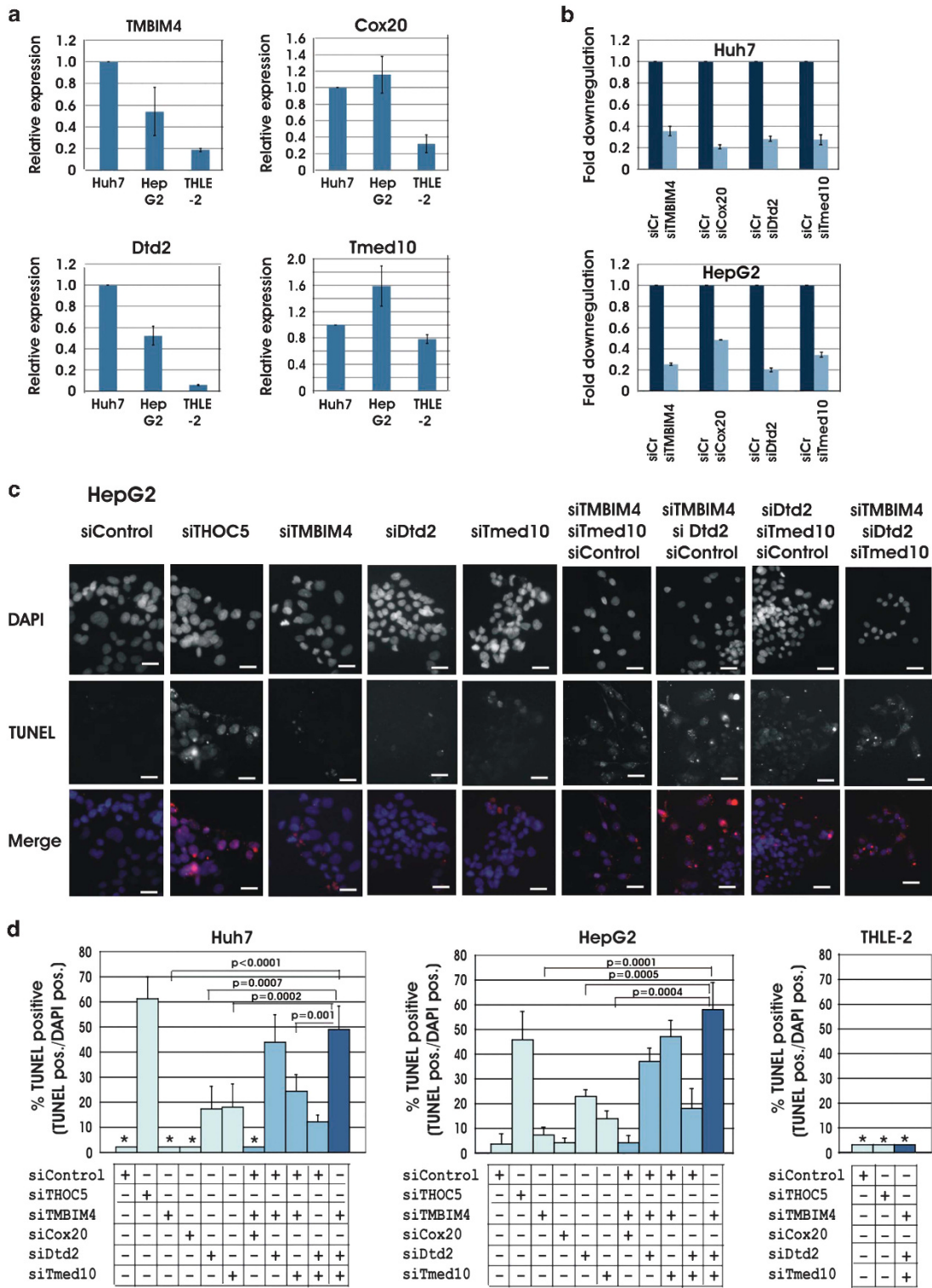


Figure 3. Depletion of a set of THOC5 target genes synergistically induced cell death of HCC. **(a)** Relative expression levels of TMBIM4, Cox20, Dtd2 and Tmed10 in Huh7, HepG2 or THLE-2 cells were measured by qRT-PCR as described in Figure 1. Numbers are mean \pm s.d. Three independent experiments were performed. **(b–d)** Control siRNA and siRNA against THOC5 (FMIP, sc-105364), TMBIM4, Cox20, Dtd2 and/or Tmed10 (total 25 pmol of siRNA for each transfection) were transfected in Huh7, HepG2 or THLE-2 cells as indicated in the figure, and 3 days after transfection, RNAs were isolated and supplied for corresponding siRNA-specific RT-PCR. Numbers are mean \pm s.d. **(b)**, or cells were stained with TUNEL and DAPI. Three independent experiments were performed and representative images are shown. Bars represent 50 μ m **(c)**. Percent of TUNEL-positive cells per DAPI-positive cells measured from 200 cells for each preparation from three independent experiments **(d)**. Numbers are mean \pm s.d. *P* value: *t*-test. * < 3%.

data imply that the depletion of a subset of fine tuning proteins, such as TMBIM4, Tmed10 and Dtd2 in HCC synergistically induces cell death.

We next examined the level of THOC5 expression in primary HCCs from patients.

Elevated THOC5 expression in human primary HCC

To examine THOC5 expression in human control liver (8 samples), liver carrying steatosis (3 samples) and hepatitis (5 samples) and 23 HCC samples (5 × differentiation grading 1 (G1) samples, 17 × differentiation grading 2 (G2) samples and 1 × differentiation

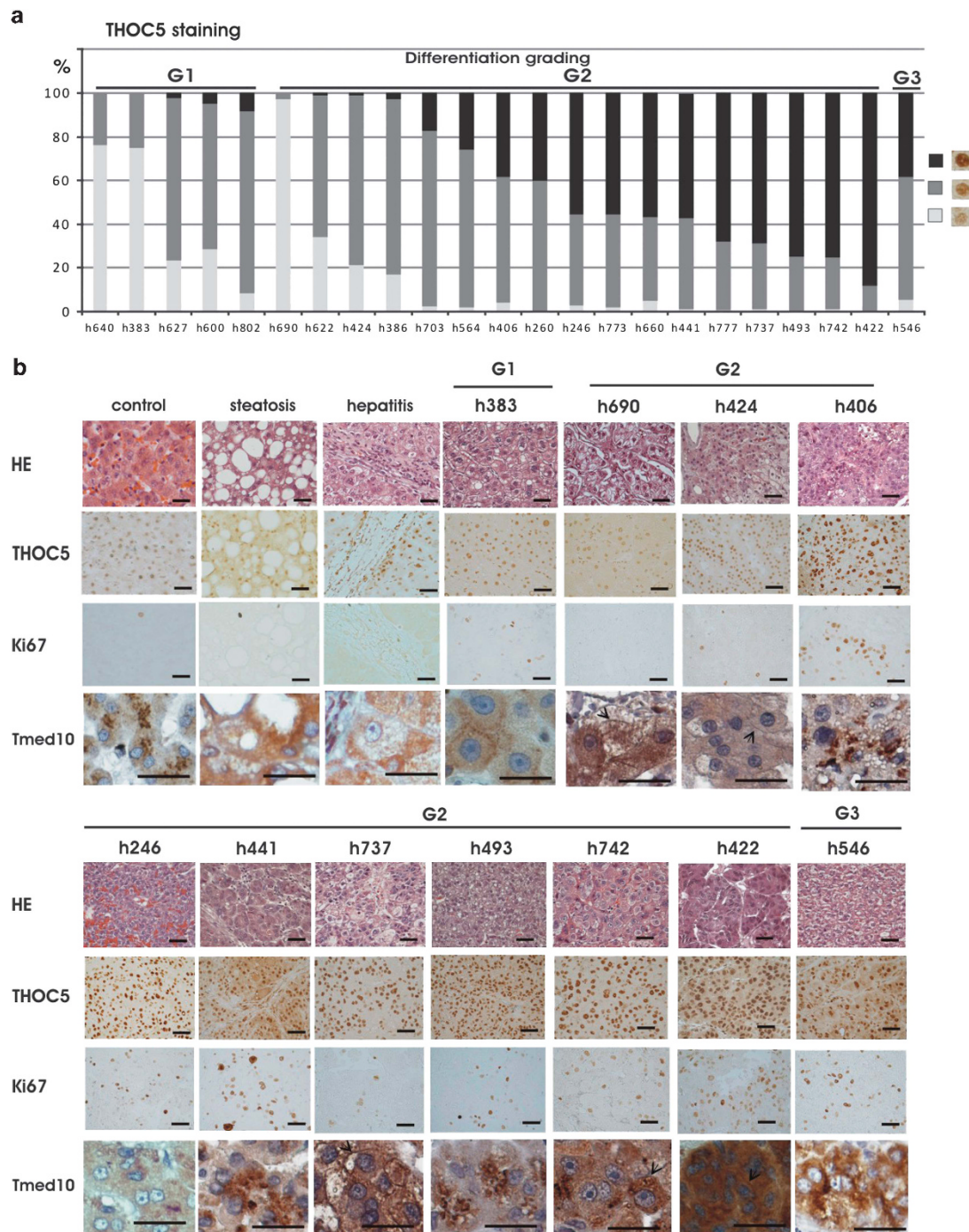


Figure 4. Enhanced expression of THOC5 in human primary HCC. Paraffin sections from 23 HCC samples, 8 control liver, 3 steatosis and 5 hepatitis samples were stained with HE, and with THOC5- and Ki67-specific antibody as detailed previously.²⁷ **(a)** Reciprocal pixel intensity of THOC5. To quantitate the intensity of THOC5 staining, reciprocal pixel intensity was determined by subtracting THOC5 intensity (as measured by the mean intensity function in the Nikon NIS elements D 3.0 Software) in 200 nuclei from the maximum pixel intensity in white unstained area from each HCC patient. Percent of light (light: reciprocal intensity 10–40), medium (med.: reciprocal intensity 40–70) or dark staining (dark: reciprocal intensity > 70) nuclei are shown. **(b)** HE-, THOC5-, Ki67 (Thermo Scientific)- and Tmed10 (Sigma, München, Germany)-specific immunohistochemical staining of samples from control liver (control), steatosis (steatosis) and hepatitis (hepatitis) liver and primary 11 HCC (representative images were shown). All images are shown in Supplementary Figure 1. Arrow heads: Tmed10 localization at plasma membrane. Bars represent 50 μ m. HE, hematoxylin-eosin.

grading 3 (G3) sample) from untreated HCC patients (Amsbio, Oxfordshire, UK, Indivumed GmbH, Hamburg, Germany), we generated paraffin sections and stained hematoxylin-eosin and THOC5-specific immunohistochemistry using a monoclonal antibody against THOC5.²² In agreement with previous data, THOC5 was stained mainly in the nuclei at different intensities (Figures 4a and b). Enhanced THOC5 expression was observed in 78% of differentiation grading 2 (G2) and G3 human HCC, but not in all G1 HCC. To quantitate the intensity of THOC5 staining, reciprocal pixel intensity of THOC5 staining in 200 nuclei of HCC from each patient was measured using Nikon NIS elements D 3.0 software (Tokyo, Japan). Percent of light (light: reciprocal intensity 10–40), medium (med.: reciprocal intensity 40–70) or dark staining (dark: reciprocal intensity 70 <) of nuclei is shown in Figure 4a. The same samples were stained with proliferation marker, Ki67-specific antibody. All samples that weakly expressed THOC5 contained few Ki67-positive cells (Figure 4b), suggesting that most HCC cells at low THOC5 expression level are in the G0 phase of the cell cycle. Figure 4b show representative images from three samples from weak THOC5 expression (< 10% dark staining), four samples from middle expression (10–60% dark expression) and four samples from THOC5 high expression (> 60% dark staining). All other samples are shown in Supplementary Figure 1. Notably, the THOC5 mRNA expression level in 379 HCC patients in cBioPortal^{18,19} is negatively correlated with patient survival (Spearman correlation, –0.140). In addition, THOC5 is expressed at a lower level in normal liver, steatosis or hepatitis liver than in HCC (Figure 4b, Supplementary Figure). These data suggest that THOC5 expression *per se* is a potential biomarker of HCC.

We further examined protein expression of the THOC5 target gene, Tmed10, in primary HCC.

THOC5 target, Tmed10, is expressed in enhanced THOC5-expressing primary HCC

Tmed10 was primarily detected as spots at perinuclear spaces in normal liver (Figure 4b, Supplementary Figure 1), suggesting that it may accumulate in or near the Golgi apparatus. A similar subcellular localization of Tmed10 was observed in Huh7 cells (data not shown). In steatosis and hepatitis liver, Tmed10 was stained weakly and diffusely in the cytoplasm, suggesting that pathological stress caused dissociation from perinuclear spaces. We further examined Tmed10 localization and expression in HCCs.

In most HCC samples, an elevated level of Tmed10 was observed in the cytoplasm and/or also at the plasma membrane (Figures 4b, h690, h424, h737, h742, h422, arrow heads), suggesting that THOC5 and Tmed10 may serve as novel biomarkers for a subset of HCC. Tmed10 is a type 1 transmembrane protein, and it consists of a 32 amino acid-long putative signal peptide, a 153 amino acid-long extracellular domain with one potential N-glycosylation site, a 21 amino acid-long transmembrane domain and a 13 amino acid-long intracellular domain. Whether Tmed10 is expressed on the outer surface of cells still, however, remains to be studied.

mRNA processing, including 5' and 3' processing, splicing and mRNA export, is tightly regulated by a complex RNA–protein network that is essential for the maintenance of cellular and tissue homeostasis. Given that depletion of one of the RNA processing regulators influences the expression of a set of genes, it is possible that these regulators might serve as a useful target molecule for cancer therapy. Evidence has accumulated that in cancer cells, these proteins are dysregulated and pre-mRNAs are alternatively spliced and/or cleaved at the 3'-end. For example, it has been recently reported that splicing kinases are dysregulated and participate in tumorigenesis.²³ Furthermore, the driver mutation of genes that encode splicing factors,²⁴ such as splicing factor 3B1 (SF3B1) or serine/arginine-rich splicing factor 2 (SRSF2) were found in several cancer cells. Indeed, the depletion of SF3B1 caused

apoptosis of breast cancer cells.²⁴ We have recently shown that THOC5 has a role in 3' end cleavage and export of a subset of mRNAs.^{11,13} In normal fibroblasts, hepatocytes or macrophages, the depletion of THOC5 does not induce cell death,^{12–14} however, here we show that THOC5 is required for HCC survival. These facts provide new opportunities for HCC therapy by depleting RNA processing proteins, or a set of their target genes. Furthermore, developing small-molecule inhibitors against Dtd2 or Tmed10 may provide opportunities for more selective anticancer strategies.

In conclusion, THOC5, a member of the mRNA export complex, is highly expressed in HCC and is required for the survival of HCC. The depletion of a single THOC5 target gene did not result in a relevant induction of cell death in HCC cells. However, the depletion of several THOC5 target genes together synergistically induced cell death of HCC cells. These data suggest that targeting multiple THOC5-dependent genes may be a useful tool for therapy of HCC.

Ethics statement

Research involving human subjects in this study was conducted in compliance with the Helsinki Declaration and all patients provided informed consent. The use of human tissue was approved and was performed in compliance with data protection regulations regarding the anonymization of the samples. All our studies are approved by the local ethics committee Ärztekammer Hamburg. Tumor samples of HCC patients and control liver specimen from patients treated at the University Medical Center Hamburg Eppendorf, Department of Hepatobiliary and Transplant Surgery were stored at Indivumed (Hamburg, Germany) following the Indivumed Standard of Biobanking (<http://www.indivumed.com>).

ABBREVIATIONS

THOC5, suppressors of the transcriptional defects of hpr1 delta by overexpression; HCC, hepatocellular carcinoma; GFP, green fluorescent protein; HE, hematoxylin-eosin; DAPI, 4',6-Diamidin-2-phenylindole; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank C Bruce Boschek for critically reading the manuscript and Iris Albers and Annika Hamm for technical assistance. SS and DDHT contributed equally to this work. This research was supported by Deutsche Krebshilfe (111153), DFG Ta-111/13-1, and PhD program Molecular Medicine and Leistungsorientierte Mittelvergabe with Frauenfaktor from MHH.

REFERENCES

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69–90.
- Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene* 2010; **29**: 4989–5005.
- Ferrin G, Aguilar-Melero P, Rodriguez-Peralvarez M, Montero-Alvarez JL, de la Mata M. Biomarkers for hepatocellular carcinoma: diagnostic and therapeutic utility. *Hepat Med* 2015; **7**: 1–10.
- Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S *et al*. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015; **47**: 505–511.
- Masuda S, Das R, Cheng H, Hurt E, Dorman N, Reed R. Recruitment of the human TREX complex to mRNA during splicing. *Genes Dev* 2005; **19**: 1512–1517.

- 6 Rehwinkel J, Herold A, Gari K, Kocher T, Rode M, Ciccarelli FL *et al*. Genome-wide analysis of mRNAs regulated by the THO complex in *Drosophila melanogaster*. *Nat Struct Mol Biol* 2004; **11**: 558–566.
- 7 Tran DD, Koch A, Tamura T. THOC5, a member of the mRNA export complex: a novel link between mRNA export machinery and signal transduction pathways in cell proliferation and differentiation. *Cell Commun Signal* 2014; **12**: 3.
- 8 Chavez S, Garcia-Rubio M, Prado F, Aguilera A. Hpr1 is preferentially required for transcription of either long or G+C-rich DNA sequences in *Saccharomyces cerevisiae*. *Mol Cell Biol* 2001; **21**: 7054–7064.
- 9 Jimeno S, Aguilera A. The THO complex as a key mRNP biogenesis factor in development and cell differentiation. *J Biol* 2010; **9**: 6.
- 10 Jimeno S, Rondon AG, Luna R, Aguilera A. The yeast THO complex and mRNA export factors link RNA metabolism with transcription and genome instability. *EMBO J* 2002; **21**: 3526–3535.
- 11 Tran DD, Saran S, Williamson AJ, Pierce A, Dittrich-Breiholz O, Wiehlmann L *et al*. THOC5 controls 3'end-processing of immediate early genes via interaction with polyadenylation specific factor 100 (CPSF100). *Nucleic Acids Res* 2014; **42**: 12249–12260.
- 12 Mancini A, Niemann-Seyde SC, Pankow R, El Bounkari O, Klebba-Farber S, Koch A *et al*. THOC5/FMIP, an mRNA export TREX complex protein, is essential for hematopoietic primitive cell survival in vivo. *BMC Biol* 2010; **8**: 1.
- 13 Guria A, Tran DD, Ramachandran S, Koch A, El Bounkari O, Dutta P *et al*. Identification of mRNAs that are spliced but not exported to the cytoplasm in the absence of THOC5 in mouse embryo fibroblasts. *RNA* 2011; **17**: 1048–1056.
- 14 Tran DD, Saran S, Dittrich-Breiholz O, Williamson AJ, Klebba-Farber S, Koch A *et al*. Transcriptional regulation of immediate-early gene response by THOC5, a member of mRNA export complex, contributes to the M-CSF-induced macrophage differentiation. *Cell Death Dis* 2013; **4**: e879.
- 15 Tamura T, Mancini A, Joos H, Koch A, Hakim C, Dumanski J *et al*. FMIP, a novel Fms-interacting protein, affects granulocyte/macrophage differentiation. *Oncogene* 1999; **18**: 6488–6495.
- 16 Pierce A, Carney L, Hamza HG, Griffiths JR, Zhang L, Whetton BA *et al*. THOC5 spliceosome protein: a target for leukaemogenic tyrosine kinases that affects inositol lipid turnover. *Br J Haematol* 2008; **141**: 641–650.
- 17 Griaud F, Pierce A, Gonzalez Sanchez MB, Scott M, Abraham SA, Holyoake TL *et al*. A pathway from leukemogenic oncogenes and stem cell chemokines to RNA processing via THOC5. *Leukemia* 2013; **27**: 932–940.
- 18 Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA *et al*. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012; **2**: 401–404.
- 19 Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013; **6**: pl1.
- 20 Panagopoulos I, Bjerkehagen B, Gorunova L, Berner JM, Boye K, Heim S. Several fusion genes identified by whole transcriptome sequencing in a spindle cell sarcoma with rearrangements of chromosome arm 12q and MDM2 amplification. *Int J Oncol* 2014; **45**: 1829–1836.
- 21 Gubser C, Bergamaschi D, Hollinshead M, Lu X, van Kuppeveld FJ, Smith GL. A new inhibitor of apoptosis from vaccinia virus and eukaryotes. *PLoS Pathog* 2007; **3**: e17.
- 22 Mancini A, Koch A, Whetton AD, Tamura T. The M-CSF receptor substrate and interacting protein FMIP is governed in its subcellular localization by protein kinase C-mediated phosphorylation, and thereby potentiates M-CSF-mediated differentiation. *Oncogene* 2004; **23**: 6581–6589.
- 23 Corkery DP, Holly AC, Lahsae S, Dellaire G. Connecting the speckles: Splicing kinases and their role in tumorigenesis and treatment response. *Nucleus* 2015; **6**: 279–288.
- 24 Maguire SL, Leonidou A, Wai P, Marchio C, Ng CK, Sapino A *et al*. SF3B1 mutations constitute a novel therapeutic target in breast cancer. *J Pathol* 2015; **235**: 571–580.
- 25 Mancini A, El Bounkari O, Norrenbrock AF, Scherr M, Schaefer D, Eder M *et al*. FMIP controls the adipocyte lineage commitment of C2C12 cells by downmodulation of C/EBP alpha. *Oncogene* 2007; **26**: 1020–1027.
- 26 Koch A, Saran S, Tran D, Klebba-Farber S, Thiesler H, Sewald K *et al*. Murine precision-cut liver slices (PCLS): a new tool for studying tumor microenvironments and cell signaling ex vivo. *Cell Commun Signal* 2014; **12**: 73.
- 27 Saran S, Tran DD, Klebba-Farber S, Moran-Losada P, Wiehlmann L, Koch A *et al*. THOC5, a member of the mRNA export complex, contributes to processing of a subset of wingless/integrated (Wnt) target mRNAs and integrity of the gut epithelial barrier. *BMC Cell Biol* 2013; **14**: 51.

Supplementary Information accompanies this paper on the Oncogene website (<http://www.nature.com/onc>)