BMB
Reports

BMB Rep. 2024; 57(2): 98-103
www.bmbreports.org

Loss of hepatic *Sirt7* accelerates diethylnitrosamine (DEN)-induced formation of hepatocellular carcinoma by impairing DNA damage repair

Yuna Kim[1,#], Baeki E. Kang[2,#], Karim Gariani[3], Joanna Gariani[4], Junguee Lee[5], Hyun-Jin Kim[2], Chang-Woo Lee[1], Kristina Schoonjans[6], Johan Auwerx[6] & Dongryeol Ryu[1,7,]*

[1]Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 16419, [2]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon 16419, Korea, [3]Service of Endocrinology, Diabetes, Nutrition and Therapeutic Patient Education, Geneva University Hospitals, Geneva 1205, [4]Department of Radiology, Hirslanden Grangettes Clinic, Geneva 1224, Switzerland, [5]Department of Pathology, Konyang University, Daejeon 35365, Korea, [6]Institute of Bioengineering, Ecole Polytechnique Federale de Lausanne, Lausanne 1015, Switzerland, [7]Department of Biomedical Science and Engineering, Gwangju Institute of Science and Technology, Gwangju 61005, Korea

The mammalian sirtuin family (SIRT1–SIRT7) has shown diverse biological roles in the regulation and maintenance of genome stability under genotoxic stress. SIRT7, one of the least studied sirtuin, has been demonstrated to be a key factor for DNA damage response (DDR). However, conflicting results have proposed that *Sirt7* is an oncogenic factor to promote transformation in cancer cells. To address this inconsistency, we investigated properties of SIRT7 in hepatocellular carcinoma (HCC) regulation under DNA damage and found that loss of hepatic *Sirt7* accelerated HCC progression. Specifically, the number, size, and volume of hepatic tumor colonies in diethylnitrosamine (DEN) injected *Sirt7*-deficient liver were markedly enhanced. Further, levels of HCC progression markers and pro-inflammatory cytokines were significantly elevated in the absence of hepatic *Sirt7*, unlike those in the control. In chromatin, SIRT7 was stabilized and colocalized to damage site by inhibiting the induction of γH2AX under DNA damage. Together, our findings suggest that SIRT7 is a crucial factor for DNA damage repair and that hepatic loss-of-*Sirt7* can promote genomic instability and accelerate HCC development, unlike early studies describing that *Sirt7* is an oncogenic factor. [BMB Reports 2024; 57(2): 98-103]

*Corresponding author. Tel: +82-62-715-5374; Fax: +82-62-715-5309; E-mail: dryu@gist.ac.kr
#These authors contributed equally to this work.

https://doi.org/10.5483/BMBRep.2023-0187

Received 9 October 2023, Revised 16 November 2023, Accepted 7 December 2023, Published online 31 January 2024

Keywords: DNA damage, Hepatocellular carcinoma, Liver cancer, SIRT7, Sirtuins

INTRODUCTION

Globally, liver cancer is the sixth most common cancer and the third leading cause of cancer fatalities (1). Hepatocellular carcinoma (HCC), the major histological subtype of primary liver cancers, frequently arises as a result of chronic injury and inflammation that can promote DNA damage and chromosomal instability (2) by initiating signaling cascades known as DNA damage response (DDR), DNA repair, and cell cycle arrest, ultimately leading to cell death or senescence (3-5).

The mammalian sirtuin family (SIRT1–SIRT7) has diverse biological roles. Genome stability regulation under genotoxic stress is a key function of sirtuins (6, 7). SIRT7, the least studied sirtuin, resides in the nucleus along with SIRT1 and SIRT6 (7, 8), wherein it exhibits catalytic activities as a selective histone H3 lysine 18 (H3K18Ac) deacetylase at DNA damage sites towards proteins involved in DNA damage and repair (9-11). In chromatin, SIRT7 also functions as a desuccinylase of histone H3 lysine 122 and a deglutarylase of histone H4 lysine 91 to promote chromatin compaction necessary for DNA damage repair (8, 9).

DDR actively participates in HCC carcinogenesis. Notably, various HCC-associated risk factors can promote DNA damage, formation of DNA adducts, and chromosomal aberrations (10). Hence, alterations in DDR pathways may trigger hepatocarcinogenesis and facilitate advanced HCC progression. *Sirt7* is generally considered as an oncogene in HCC as it is upregulated in human HCC samples. Its expression increased with increasing tumor grade (11). However, effects of SIRT7 deficiency on DNA damage-induced HCC development and progression *in vivo* remain unclear. Related molecular mechanisms have not been elucidated either.

Diethylnitrosamine (DEN) is a DNA-alkylating carcinogen widely used to study HCC progression. It mimics human HCC by stimulating inflammatory responses and hyperproliferation

ISSN: 1976-670X (electronic edition)

(12). Therefore, the objective of this study was to investigate pathological characteristics of SIRT7 in the regulation of hepatic tumorigenesis driven by DEN-induced DNA damage in a liver-specific *Sirt7* null mouse model.

RESULTS

Sirt7 depletion in liver accelerates tumorigenesis in DEN-induced hepatocarcinogenesis

To investigate whether alteration of *Sirt7* plays a role in hepatocarcinogenesis and its involvement in HCC progression, we first generated a *Sirt7* liver-specific knockout mouse model ($Sirt7^{hep-/-}$) and exposed it to chronic DEN treatment (Fig. 1A). Ten weeks after DEN administration, total body and liver weights did not differ between the two genotypes (Fig. 1B). However, the ratio of liver to body weight was slightly heavier in $Sirt7^{hep-/-}$ mice than in control mice possibly because of increased number and size of HCC lesions. This was further validated by gross anatomy analysis (Fig. 1C and Supplementary Fig. 1A). In addition, observable numbers of tumor nodules increased with tumor volumes in DEN-induced $Sirt7^{hep-/-}$ mice compared to those in their wild-type (WT) littermates, as demonstrated by liver micro-CT images (Fig. 1D, E and Supplementary Fig. 1B, C). Collectively, these phenotypic changes suggest that deficiency of *Sirt7* in the liver can potentiate the promotion of hepatic tumorigenesis driven by DEN-induced DNA damage.

Hepatic *Sirt7*-knockout mice exhibit hallmarks of HCC progression

To further evaluate effects of *Sirt7* deletion on HCC onset and progression, histological analysis was performed for liver tumors. $Sirt7^{hep-/-}$ mice showed more malignant or pre-malignant nodular in cancerous lesions (Fig. 2A), with increased central-to-portal bridging fibrosis in non-cancerous liver lesions compared to that in WT littermates (Supplementary Fig. 2A, B). In histological analysis of $Sirt7^{hep-/-}$ livers, levels of Ki-67 (a proliferation marker) and alpha fetoprotein (AFP, an HCC marker) were elevated (Fig. 2B, C). Consistently, serum AFP levels exhibited a significant increase (Fig. 2D). Gene expression levels of hallmarks of HCC progression, including *Afp*, *Kitl*, *Vegf*, *Pdgfpβ*, and *Ki67*, were also markedly elevated upon *Sirt7* deletion (Fig. 2E). Similarly, pro-inflammatory cytokines known to play important roles in HCC development were also increased in $Sirt7^{hep-/-}$ mice compared to those in WT mice (Supplementary Fig. 2C, D). Furthermore, expression levels of DNA damage related proteins (Trp53 and CDKN1A/p21) and proliferation makers (PCNA and AFP) were higher in livers of *Sirt7*-depleted mice compared with those in WT mice (Fig. 2F). Taken together, these findings suggest that *Sirt7* depletion following DEN-induced DNA damage can lead to pro-tumorigenic inflammation in the liver, eventually accelerating hepatic carcinogenesis.

Sirt7 expression is positively correlated with DNA repair gene-set

To clarify the pathological involvement of *Sirt7* based on our *in vivo* findings, we performed gene set enrichment analysis (GSEA; www.broadinstitute.org/gsea) using hepatic transcriptomes of BXD mouse strains (13). Results of GO and KEGG enrichment analysis confirmed that *Sirt7* expression was highly involved in the DNA damage pathway (Fig. 3A) and positively correlated with DNA damage repair-related gene sets (Fig. 3B,

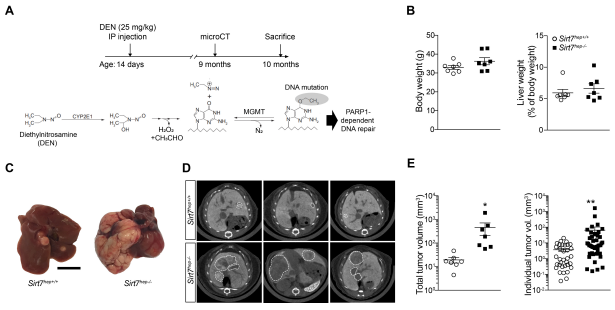

Fig. 1. Loss of hepatic *Sirt7* promotes hepatic tumorigenesis in DEN-injected mice. (A) Schematic diagram showing diethylnitrosamine (DEN)-induced hepatocarcinogenesis mouse model. MGMT: O^6-Methylguanine-DNA Methyltransferase. (B) Body and normalized liver weights at sacrifice (n = 7 per group). (C) Representative images of livers from 10-month-old $Sirt7^{hep+/+}$ and $Sirt7^{hep-/-}$ mice. (D) Liver micro-CT images captured from 9-month-old $Sirt7^{hep+/+}$ and $Sirt7^{hep-/-}$ mice. (E) Quantitative analysis of hepatic tumors using micro-CT. Data in (B) and (E) represent the mean ± SEM. *P ≤ 0.05, **P ≤ 0.01, unpaired Student's *t*-test.

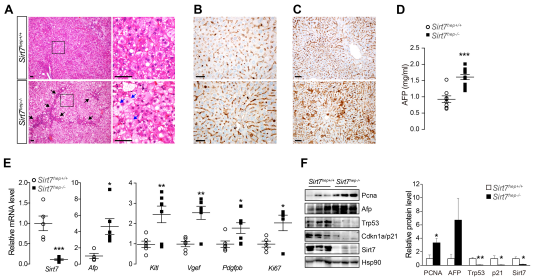

Fig. 2. Effects of hepatic loss-of-*Sirt7* in liver tumor. (A) The level of tumor development was measured by hematoxylin/eosin (H&E) staining of liver tumor obtained from $Sirt7^{hep+/+}$ and $Sirt7^{hep-/-}$ mice (10-month-old male). (B, C) Ki67 staining and Alpha-fetoprotein (AFP) and in liver tumors of $Sirt7^{hep+/+}$ and $Sirt7^{hep-/-}$ mice (10-month-old male). (D) ELISA showing serum AFP levels in $Sirt7^{hep+/+}$ and $Sirt7^{hep-/-}$ mice (10-month-old male). (E) The qRT-PCR results using tumor tissues showing hepatic expression of hallmarks of HCC progression such as *Afp*, *Kitl*, *Vegf*, *Pdgfpβ*, and *Ki67*. (F) Western blot analysis of DNA damage and tumor proliferation-related molecules, including PCNA, AFP, Trp53, and p21, was performed in $Sirt7^{hep+/+}$ and $Sirt7^{hep-/-}$ mice (10-month-old male). The quantification of western blot (right). Data in (D), (E), and (F) represent the mean ± SEM. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Unpaired Student's *t*-test.

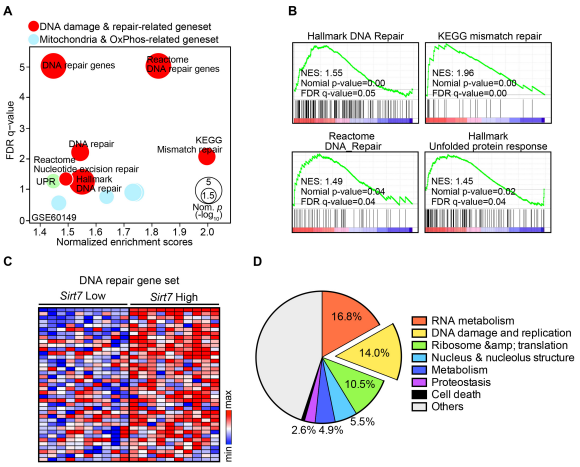

Fig. 3. *Sirt7* expression is positively correlated with the DNA repair gene-set. (A-C) GSEA results of the hepatic transcriptomes of BXD-GRP mice showing a correlation between *Sirt7* and DNA repair genes. (A) Bubble chart showing the normalized enrichment score (x-axis), false discovery rate (FDR; y-axis), and nominal P value (size of bubble; −log10). (B) GSEA plots of DNA damage repair gene sets. (C) Heat maps showing the expression of DNA repair genes in relation to *Sirt7* expression levels in livers of BXD mice. (D) Pie charts showing functional clustering of protein-protein interactions.

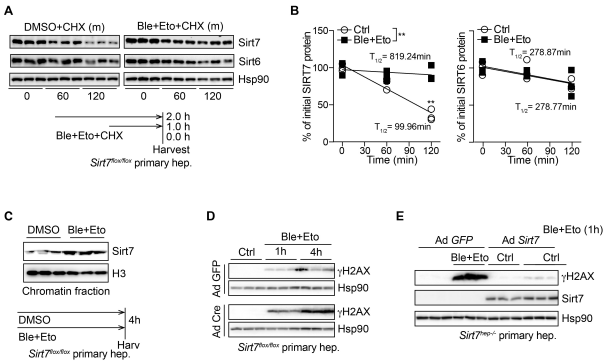

Fig. 4. SIRT7 is a crucial factor in DNA repair. (A, B) Comparison of Protein Stability of SIRT7 and SIRT6 following DNA damage induced by treatment with bleomycin and etoposide for 0, 60, or 120 min. (C) Analysis of SIRT7 localization changes following induction of DNA damage for 4 hours using chromatin fractionation. (D, E) The effect of genetic aberrations, loss and gain of *Sirt7*, upon DNA damage indicated by γH2AX in isolated $Sirt7^{flox/flox}$ primary hepatocytes. Adenovirus-mediated deletion of Cre recombinase (Ad Cre) and overexpression of SIRT7 (Ad GFP). Data are represented as mean ± SEM. **P < 0.01. Unpaired Student's *t*-test.

C and Supplementary Fig. 3A). To bolster these findings, we conducted a re-analysis of the proteomics showing potential SIRT7 interactome (14). In accordance with transcriptomic analysis, functional clustering of protein interactions revealed SIRT7's role in various biological processes such as RNA metabolism (16.8%), DNA damage and replication (14%), and ribosome and translation (10.5%) (Fig. 3D). Taken together, bioinformatics analysis strongly suggests that *Sirt7* is essential for DNA damage repair.

SIRT7 is a crucial factor for DNA damage repair

To determine whether SIRT7 was a crucial factor for DNA damage repair, we blocked *de novo* protein synthesis using cycloheximide (CHX), a protein synthesis inhibitor, to determine the half-life of SIRT7. SIRT7 degradation was delayed upon treatment with bleomycin and etoposide (Ble + Eto) which induced DNA damage, whereas no difference was detected in the expression of SIRT6 (Fig. 4A, B), a DNA double-strand break (DSB) sensor (15). Interestingly, SIRT7 degradation significantly slowed and its half-life sustained for up to 4 h under DNA damage found mostly in chromatin fractions (Fig. 4C and Supplementary Fig. 4A), indicating that DNA damage might stabilize SIRT7 and shift its localization to the chromatin. We examined whether alterations in SIRT7 had potential to participate in DNA damage repair by examining the level of γH2AX induction under conditions of DNA damage in Sirt7-floxed (Sirt7L2/L2) primary hepatocytes (Fig. 4D). It was found that deletion of SIRT7 using adenovirus-mediated Cre-recombinase (Ad Cre) significantly increased γH2AX expression compared to the control (Ad GFP). However, reconstruction of SIRT7 in SIRT7 deleted primary hepatocytes markedly suppressed γH2AX induction (Fig. 4E). These findings support SIRT7's crucial role in repairing DNA damage.

DISCUSSION

Early studies have demonstrated that Sirt7 could play a pivotal role in hepatic metabolism (16, 17). The absence of Sirt7 has been associated with the onset of non-alcoholic fatty liver disease, a prevalent chronic liver condition in developed regions and a recognized risk factor for HCC (18). Here, we investigated the effect of hepatic *Sirt7* deficiency in a mouse model of liver cancer in which HCC was driven by chronic exposure to DEN-induced DNA damage. Our results revealed a potential protective effect of *Sirt7* against genetic aberrations in chronic HCC development. Regardless of the etiology, the majority of cirrhotic livers progress to HCC and over 90% of them show chromosomal abnormalities that are present universally in HCC. In damaged cells, chromosomal defects proliferate to form dysplastic nodules, which usually transform into HCC precursors. Therefore, increased genomic instability is a hallmark of HCC. Persistent DNA damage with defects in DNA repair contributes to genomic instability and eventually accelerates tumorigenesis (19).

To ensure genome stability, DSBs must be repaired. Unrepaired DNA lesions eventually lead to cancer pathologies (20). Several processes of DNA damage repair correspond to mechanisms implicated in HCC, such as stalled DNA replication

forks by homologous recombination (HR) (21), base mismatches by mismatch repair (MMR) (22), and DBS, the most serious form of DNA damage (23), by non-homologous end joining (NHEJ) (24). The major pathway for repairing DSBs is NHEJ, which modifies broken DNA ends and ligates them together regardless of homology by deletions or insertions (25). Therefore, NHEJ is intrinsically mutagenic (26). Defects in DDR by NHEJ can lead to chromosomal instability and contribute to HCC (27).

SIRT7 appears to be a crucial player in DDR pathways as it has a histone deacetylase activity at DNA damage sites and additional catalytic activities to modify proteins involved in DNA damage and repair (9, 28). More specifically, SIRT7 displays a protective role in cancer cell survival under genotoxic stress-induced DNA damage by halting cell cycle to ensure DNA repair and by accumulating γH2AX and p53 while attenuating stress-activated protein kinases (p38 and JNK) to maintain genomic integrity (29). Of these, DDR-related genes are key to maintaining genomic stability. Loss of DDR can result in the onset and progression of cancer (30). Of note, Vazquez *et al*. have found that *Sirt7*$^{-/-}$ cells with accumulating DNA mutations and replication stress can result in genome instability, leading to compromised NHEJ (28). Consistent with these findings, we also observed that hepatic *Sirt7*$^{-/-}$ mice injected with DEN displayed accelerated HCC development compared to WT littermates while proteins related to DDR were significantly downregulated with liver primary *Sirt7*$^{-/-}$ cells showing higher proliferative activities. In chromatin, we witnessed SIRT7 recruitment to form discrete nuclear foci that instantly colocalized with γH2AX, which reflected the induction of DSBs, triggering recruitment of various DNA-damage repair proteins to damaged sites (31) after DNA damage induction. Additionally, HCC progression in *Sirt7*$^{hep\,-/-}$ mice caused the development of more visible numbers of tumor nodules with higher tumor volumes than that in WT mice, strongly suggesting that loss of *Sirt7* upon DNA damage could affect tumor growth. This implies a higher susceptibility to carcinogenesis under *Sirt7* deletion, which infers a significant contribution of SIRT7 in DSB pathways to protect tumorigenesis.

Inconsistent with such findings, however, increased SIRT7 expression is frequently observed in various human cancers (11, 32). In particular, SIRT7 was significantly upregulated and positively associated with HCC grade in a large cohort of patients (33). Zhao *et al*. have also observed that both *Sirt7* mRNA and protein levels are significantly upregulated in most HCC tissues compared with those in adjacent non-tumoral liver tissues (34). Furthermore, *Sirt7* expression is significantly correlated with poor overall survival rate and relatively lower in normal liver and primary human hepatocytes (35). These data suggest that *Sirt7* might act as an oncogene during HCC development. Furthermore, SIRT7 is a selective deacetylase of H3K18, which is an epigenetic biomarker of aggressive tumors (36).

In the present study, we described a DNA damage induced *Sirt7* deficiency HCC mouse model, in which a gene that participated in DDR probably through the NHEJ pathway upon DEN exposure, caused widespread chromosomal aberrations, disrupted cell cycle checkpoints, and accelerated liver carcinogenesis. The importance of SIRT7 in DNA damage repair, as identified in our study, suggests that this enzyme might function as a tumor suppressor.

To the best of our knowledge, a plausible explanation for these paradoxical results is that SIRT7 might have contradictory effects on cancer initiation and progression. SIRT7 is an important regulator of cancer cell growth and survival. It might act as a molecular bridge between genome stability and cancer progression. However, detailed mechanisms underlying these opposing effects remain largely unknown. They require further investigations. More systematic research is required to outline how SIRT7 function might change across cancer initiation and development at molecular and physiological levels. Such studies will enable us to develop novel therapeutic targets for clinical applications in HCC (37-39).

In conclusion, our findings indicate that Sirt7 plays a dual role in the liver. It appears to actively participate in the control of hepatic energy metabolism by directly influencing the expression of mitochondrial genes and managing endoplasmic reticulum (ER) stress (16, 17). Sirt7 also appears to be involved in preserving genomic stability in the liver, a tissue particularly vulnerable to the impact of diverse toxicants. However, further in-depth mechanistic studies are necessary.

MATERIALS AND METHODS

Animal experiments

All animal experiments were performed in accordance with the Swiss and EU ethical guidelines and were approved by the local animal experimentation committee of Canton de Vaud under license #2868 and also by the Institutional Animal Care and Use Committee (IACUC) of Sungkyunkwan University School of Medicine (SUSM) (code/SKKUIACUC 2021-07-47-1) and Gwangju Institute of Science and Technology (GIST-2023-027). Details of the experimental protocols are provided in the Supplementary Materials.

Generation of *Sirt7*$^{hep\,-/-}$ mice and tumorigenesis

Liver-specific Sirt7 knockout mice (*Sirt7*$^{hep\,-/-}$) were generated as previously specified (17). To initiate the development of liver tumors, male mice of the C57BL/6 strain, specifically *Sirt7*$^{hep\,-/-}$ and *Sirt7*$^{hep+/+}$ littermates, were subjected to intraperitoneal injections of DEN (Sigma) at a dose of 25 mg/kg body weight on day 14 postpartum as described (40, 41).

All mice were monitored weekly for changes in weight and activity. Nine months following the injection of DEN, liver tumor volumes and numbers induced by DEN were assessed utilizing micro-CT analysis. Ten months following DEN injection, all mice were humanely euthanized, and liver and blood tissues were collected for further analysis.

Primary mouse hepatocyte culture

In accordance with established procedures (17), primary liver cells from wild type C57BL/6J, S mice aged seven to nine weeks were procured. Subsequent to isolation, these cells were cultured in Media 199 with a composition of 4.5 g/L glucose, 10% fetal bovine serum, 0.1 mM NEAA, 25 mM HEPES, 100 nM dexamethasone, and 50 μg/ml gentamicin. The cultured cells were maintained at 37°C in a 5% CO_2 environment, as outlined in a previously published study. Adenoviruses were prepared according to the methods described in previous studies (17, 42), and administered to primary hepatocytes six hours after they were seeded.

Western blots

Western blot analyses were conducted using 50-100 μg of protein from whole cell lysates and 5-30 μg of protein from subcellular fractions, following established procedures detailed in previous publications (40, 41). The antibodies used in these analyses are listed in Supplementary Table 2.

Histology

Freshly harvested liver tissues were fixed, embedded, and sliced into 5 μm thick sections. Briefly, paraffin sections were first deparaffinized and then hydrated to develop in diaminobenzidine solution under a microscope and counterstained with hematoxylin and eosin (H&E) (17, 43) and immunostaining was performed using Ki67 (1:100) and AFP (1:100) primary monoclonal antibodies.

Total RNA extraction and real-time PCR

As described (42), total RNA was extracted using Trizol reagent (Invitrogen, USA) and was reverse-transcribed into complementary DNA with random hexamer primer (Thermo Fisher Scientific, USA). The qRT-PCR was performed in triplicates using QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, USA) according to the manufacturer's instructions. The primers used for qRT-PCR were listed in supplementary data.

Bioinformatics analysis

All bioinformatics analysis and visualization were conducted as previously described (44, 45). All raw transcriptomic data are publicly available on the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) under the accession numbers GSE48452 and GSE24031, or GeneNetwork (www.genenetwork.org). Heat maps were constructed using GENE-E software (The Broad Institute, www.broadinstitute.org/cancer/software/GENE-E/). Principal component analysis and construction of corrgrams and correlation networks were performed using functions and packages in R (www.r-project.org). The depth of shading in the correlation matrices indicates the magnitude of the correlation (Pearson's r). Positive and negative correlations within the corrgram and correlation network are represented in blue and red, respectively. Only correlations with $P < 1E\text{-}05$ are displayed in the correlation network.

Statistical analysis

Statistical analyses were performed using Prism (version 8.0; GraphPad Software Inc.) and R software. The significance of the differences between the two groups was determined using unpaired two-tailed Student's t-tests. To compare multiple groups, we applied a one-way analysis of variance (ANOVA) with a post-hoc Bonferroni test. The results are presented as mean ± standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

ACKNOWLEDGEMENTS

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (NRF-2021R1A6A3A13044725 to Y.K.; 2020R1A2C2010964, 2022K2A9A1A06091879, 2023R1A2C3006220, RS-2023-00283539, and RS-2023-00261370 to D.R.). D.R. was supported by a "GIST Research Institute (GRI) IIBR" grant funded by the GIST in 2023.

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Sung H, Ferlay J, Siegel RL et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71, 209-249
2. Buitrago-Molina LE, Marhenke S, Longerich T et al (2013) The degree of liver injury determines the role of p21 in liver regeneration and hepatocarcinogenesis in mice. Hepatology 58, 1143-1152
3. Fortini P, Ferretti C and Dogliotti E (2013) The response to DNA damage during differentiation: pathways and consequences. Mutat Res 743-744, 160-168
4. Lee YH, Kuo CY, Stark JM, Shih HM and Ann DK (2013) HP1 promotes tumor suppressor BRCA1 functions during the DNA damage response. Nucleic Acids Res 41, 5784-5798
5. Ogara MF, Sirkin PF, Carcagno AL et al (2013) Chromatin relaxation-mediated induction of p19INK4d increases the ability of cells to repair damaged DNA. PLoS One 8, e61143
6. Chalkiadaki A and Guarente L (2015) The multifaceted functions of sirtuins in cancer. Nat Rev Cancer 15, 608-624
7. Cha YI and Kim HS (2013) Emerging role of sirtuins on tumorigenesis: possible link between aging and cancer. BMB Rep 46, 429-438
8. Bao X, Liu Z, Zhang W et al (2019) Glutarylation of histone H4 lysine 91 regulates chromatin dynamics. Mol Cell 76, 660-675 e669
9. Li L, Shi L, Yang S et al (2016) SIRT7 is a histone desuccinylase that functionally links to chromatin compaction and genome stability. Nat Commun 7, 12235
10. Guo X, Yanna, Ma X et al (2011) A meta-analysis of

array-CGH studies implicates antiviral immunity pathways in the development of hepatocellular carcinoma. PLoS One 6, e28404
11. Kim JK, Noh JH, Jung KH et al (2013) Sirtuin7 oncogenic potential in human hepatocellular carcinoma and its regulation by the tumor suppressors MiR-125a-5p and MiR-125b. Hepatology 57, 1055-1067
12. Vesselinovitch SD, Koka M, Mihailovich N and Rao KV (1984) Carcinogenicity of diethylnitrosamine in newborn, infant, and adult mice. J Cancer Res Clin Oncol 108, 60-65
13. Singer JB, Hill AE, Burrage LC et al (2004) Genetic dissection of complex traits with chromosome substitution strains of mice. Science 304, 445-448
14. Tsai YC, Greco TM, Boonmee A, Miteva Y and Cristea IM (2012) Functional proteomics establishes the interaction of SIRT7 with chromatin remodeling complexes and expands its role in regulation of RNA polymerase I transcription. Mol Cell Proteomics 11, 60-76
15. Onn L, Portillo M, Ilic S et al (2020) SIRT6 is a DNA double-strand break sensor. Elife 9, e51636
16. Shin J, He M, Liu Y et al (2013) SIRT7 represses Myc activity to suppress ER stress and prevent fatty liver disease. Cell Rep 5, 654-665
17. Ryu D, Jo YS, Lo Sasso G et al (2014) A SIRT7-dependent acetylation switch of GABPbeta1 controls mitochondrial function. Cell Metab 20, 856-869
18. Estes C, Razavi H, Loomba R, Younossi Z and Sanyal AJ (2018) Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 67, 123-133
19. Hanahan D and Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144, 646-674
20. Jackson SP and Bartek J (2009) The DNA-damage response in human biology and disease. Nature 461, 1071-1078
21. Chung YL and Wu ML (2013) Promyelocytic leukaemia protein links DNA damage response and repair to hepatitis B virus-related hepatocarcinogenesis. J Pathol 230, 377-387
22. Bayram S, Akkiz H, Bekar A, Akgollu E and Yildirim S (2012) The significance of Exonuclease 1 K589E polymorphism on hepatocellular carcinoma susceptibility in the Turkish population: a case-control study. Mol Biol Rep 39, 5943-5951
23. Hsu CM, Yang MD, Chang WS et al (2013) The contribution of XRCC6/Ku70 to hepatocellular carcinoma in Taiwan. Anticancer Res 33, 529-535
24. Lees-Miller SP and Meek K (2003) Repair of DNA double strand breaks by non-homologous end joining. Biochimie 85, 1161-1173
25. Lieber MR (2008) The mechanism of human nonhomologous DNA end joining. J Biol Chem 283, 1-5
26. Thompson LH and Schild D (2001) Homologous recombinational repair of DNA ensures mammalian chromosome stability. Mutat Res 477, 131-153
27. Teoh NC, Dan YY, Swisshelm K et al (2008) Defective DNA strand break repair causes chromosomal instability and accelerates liver carcinogenesis in mice. Hepatology 47, 2078-2088
28. Vazquez BN, Thackray JK, Simonet NG et al (2016) SIRT7 promotes genome integrity and modulates non-homologous end joining DNA repair. EMBO J 35, 1488-1503
29. Kiran S, Oddi V and Ramakrishna G (2015) Sirtuin 7 promotes cellular survival following genomic stress by attenuation of DNA damage, SAPK activation and p53 response. Exp Cell Res 331, 123-141
30. Jeggo PA, Pearl LH and Carr AM (2016) DNA repair, genome stability and cancer: a historical perspective. Nat Rev Cancer 16, 35-42
31. Nakamura AJ, Rao VA, Pommier Y and Bonner WM (2010) The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks. Cell Cycle 9, 389-397
32. Wang HL, Lu RQ, Xie SH et al (2015) SIRT7 exhibits oncogenic potential in human ovarian cancer cells. Asian Pac J Cancer Prec 16, 3573-3577
33. Kim JK, Noh JH, Jung KH et al (2013) Sirtuin7 oncogenic potential in human hepatocellular carcinoma and its regulation by the tumor suppressors MiR-125a-5p and MiR-125b. Hepatology 57, 1055-1067
34. Zhao J, Wozniak A, Adams A et al (2019) SIRT7 regulates hepatocellular carcinoma response to therapy by altering the p53-dependent cell death pathway. J Exp Clin Cancer Res 38, 252
35. Zhao J, Wozniak A, Adams A et al (2019) SIRT7 regulates hepatocellular carcinoma response to therapy by altering the p53-dependent cell death pathway. J Exp Clin Cancer Res 38, 252
36. Seligson DB, Horvath S, McBrian MA et al (2009) Global levels of histone modifications predict prognosis in different cancers. Am J Pathol 174, 1619-1628
37. Kim DH, Kim MJ, Kim NY et al (2022) DN200434, an orally available inverse agonist of estrogen-related receptor γ, induces ferroptosis in sorafenib-resistant hepatocellular carcinoma. BMB Rep 55, 547-552
38. Moon B, Park M, Cho SH et al (2022) Synergistic antitumor activity of sorafenib and MG149 in hepatocellular carcinoma cells. BMB Rep 55, 506-511
39. Lee S, Byun JK, Kim NY et al (2022) Melatonin inhibits glycolysis in hepatocellular carcinoma cells by downregulating mitochondrial respiration and mTORC1 activity. BMB Rep 55, 459-464
40. Schulien I and Hasselblatt P (2021) Diethylnitrosamine-induced liver tumorigenesis in mice. Methods Cell Biol 163, 137-152
41. Shirakami Y, Gottesman ME and Blaner WS (2012) Diethylnitrosamine-induced hepatocarcinogenesis is suppressed in lecithin: retinol acyltransferase-deficient mice primarily through retinoid actions immediately after carcinogen administration. Carcinogenesis 33, 268-274
42. Yoon YS, Ryu D, Lee MW, Hong S and Koo SH (2009) Adiponectin and thiazolidinedione targets CRTC2 to regulate hepatic gluconeogenesis. Exp Mol Med 41, 577-583
43. Yamamoto H, Williams EG, Mouchiroud L et al (2011) NCoR1 is a conserved physiological modulator of muscle mass and oxidative function. Cell 147, 827-839
44. Kim J, Lee H, Jin EJ et al (2022) A microfluidic device to fabricate one-step cell bead-laden hydrogel struts for tissue engineering. Small 18, e2106487
45. Kim J, Jo Y, Cho D and Ryu D (2022) L-threonine promotes healthspan by expediting ferritin-dependent ferroptosis inhibition in C. elegans. Nat Commun 13, 6554