Identification of critical genes and biological signaling for metformin treated liver cancer

Tingting Zhang, Hongmei Guo, Letian Wang, Mengyao Wang, Hanming Gu¹

¹SHU-UTS SILC School, Shanghai University, Shanghai, China

*Corresponding author: Hanming Gu, SHU-UTS SILC School, Shanghai University, Shanghai, China

laygmp@gmail.com

Abstract

Liver cancer is a leading source of cancer-related mortality in the world. A number of studies have shown the correlation of metformin treatment with a decrease in cancer risk. However, the relevant molecules and mechanisms are not clear during the treatment. In this study, our aim is to identify the significant molecules and signaling pathways in the treatment of metformin in liver cancer cells by analyzing the RNA sequence. The GSE190076 dataset was created by performing the Illumina NovaSeq 6000 (Homo sapiens). The KEGG and GO analyses indicated that DNA synthesis and cell cycle are the main processes during the treatment of metformin. Moreover, we determined numerous genes including RRM2, CDC6, CDC45, UHRF1, ASF1B, ZWINT, PCNA, ASPM, MYC, and TK1 by using the PPI network. Therefore, our study may guide the clinical work on the treatment of liver cancer by using metformin.

Introduction

Primary liver cancer is an aggressive tumor that is one of the most common cancers in the world¹. In the United States, the annual incidence of liver cancer is increasing despite the advanced treatment, and liver cancer remains one of the most difficult cancers to cure². There are various treatments for liver cancer, which are selected according to the complex interplay of tumor stage and patient's status³.

Metformin is an essential drug for the treatment of diabetes, which is the most commonly used oral drug and is recommended as the first-line therapy for T2D patients⁴. Besides diabetes, metformin is also used in polycystic ovary diseases, diabetic nephropathy, and cardiovascular complications⁵. Most importantly, metformin indicated the anti-cancer functions through both indirect and direct effects⁶. The indirect mechanisms contribute to the regulation of blood glucose and insulin, which could affect cancer cell survival⁷. Metformin directly inhibits the NF- κ B activity to further activate the immune response to cancer cells⁸. For example, metformin enhanced the effectiveness of the anti-cancer vaccine that was regulated by the activation of T cells⁹.

Here, our present studies determined the DEGs and signaling pathways of metformin in liver cancer by analyzing the RNA-seq data. We figured out several functional DEGs and processes and created the protein-protein interaction (PPI) network. It is possible

that our understanding of the anti-cancer properties of metformin will result in advances in the treatment of liver cancer.

Methods

Data resources

The data (GSE190076) was created by using Illumina NovaSeq 6000 (Homo sapiens) (Hebei University, Number 180, Wusi east street, Baoding, Hebei Province, China). The analyzed dataset includes three groups of control liver cancer cells and three groups of liver cancer cells treated by metformin.

Data acquisition and preprocessing

The data were processed by the R package as described¹⁰⁻¹⁴. A classical t-test was performed to identify DEGs with $P < 0.01$ and fold change ≥ 1 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses

The KEGG and GO analyses were performed from the R package (clusterProfiler) and Reactome (<https://reactome.org/>). We set the $P < .05$ and gene counts > 10 as the statistically significant cutoff.

Protein-protein interaction (PPI) analysis

The PPI was constructed by using the Molecular Complex Detection (MCODE). The biological processes analysis was performed by Reactome, and $P < 0.05$ was considered as the statistically significant cutoff.

Results

Identification of DEGs of metformin treated liver cancer cells

To understand the mechanism of the effects of metformin on liver cancers, we analyzed the RNA-seq data from the metformin treated liver cancer cells. A total of 125 genes were identified with the threshold of $P < 0.001$. The top up-and down-regulated genes in

metformin treated liver cancer cells were identified by the heatmap and volcano plot (Figure 1). The top ten DEGs were listed in Table 1.

Enrichment analysis of DEGs in the metformin treated liver cancer cells

To further understand the potential biological functions of the DEGs, we introduced the KEGG and GO analyses (Figure 2). The significant items of KEGG were selected, including “Cell cycle”, “Cellular senescence”, “TGF- β signaling pathway”, “DNA replication”, and “Fanconi anemia pathway”, and “Mismatch repair”. We also identified the top ten biological processes of GO, including “Regulation of mitotic cell cycle”, “Nuclear division”, “Chromosome segregation”, “Negative regulation of cell cycle”, “DNA replication”, “Nuclear chromosome segregation”, “Sister chromatid segregation”, “Mitotic sister chromatid segregation”, “DNA-dependent DNA replication”, and “Cell cycle checkpoint signaling”. We then identified the top ten cellular components of GO, including “chromosomal region”, “spindle”, “condensed chromosome”, “chromosome, centromeric region”, “condensed chromosome, centromeric region”, “mitotic spindle”, “kinetochore”, “spindle midzone”, “DNA replication preinitiation complex”, and “CMG complex”. We identified the top ten molecular functions of GO, including “ubiquitin-like protein transferase activity”, “ubiquitin-like protein ligase activity”, “catalytic activity, acting on DNA”, “histone binding”, “helicase activity”, “single-stranded DNA binding”, “ATP-dependent activity, acting on DNA”, “DNA helicase activity”, “single-stranded DNA helicase activity”, and “DNA replication origin binding”.

Construction of PPI network in the metformin treated liver cancer cells

To further identify the potential relationship of the DEGs, we constructed the PPI network by the String and Cytoscape software. The PPI network was created by using 116 nodes and 164 edges (combined score > 0.4). The top ten genes were selected with the highest degree scores (Table 2). The String network and top clusters were shown in Figure 3. We further analyzed the PPI genes and DEGs by Reactome map (Figure 4) and selected the top ten potential biological functions including “G1/S-Specific Transcription”, “Mitotic G1 phase and G1/S transition”, “G1/S Transition”, “Cell Cycle, Mitotic”, “Cell Cycle”, “Transcription of E2F targets under negative control by DREAM complex”, “Transcriptional Regulation by E2F6”, “G0 and Early G1”, and “Activation of ATR in response to replication stress” (Supplemental Table S1).

Discussion

Metformin can inhibit hepatic gluconeogenesis and promote glucose uptake in muscles, and it also can improve the prognosis of cancer patients and block the progression of tumor^{6, 15}. Thus, metformin is currently being investigated for new anti-cancer drugs¹⁶.

Metformin is a key regulator in liver cancer by mediating the DNA replication and cell cycle through the KEGG and GO analyses. Interestingly, Sung-Hee Kim et al found metformin enhances chemo-sensitivity by decreasing DNA replication proteins in colorectal cancer cell¹⁷. Krisztina Kisfalvi et al found metformin disturbs the relationship between G protein-coupled receptor and insulin receptor signaling systems to further prevent cancer growth¹⁸. As key signaling regulators, GPCR and RGS proteins are involved in a variety of human diseases including cancer, arthritis, bone diseases, and heart diseases¹⁹⁻²⁹. It was found that low doses of metformin inhibited the DNA synthesis and tumor growth by insulin and GPCR agonists¹⁸. James Sinnott-Smith et al found metformin can inhibit the activation of mTORC1, DNA synthesis in pancreatic cancer by affecting the AMPK signaling³⁰. Metformin is capable to inhibit the proliferation of multiple myeloma cells by the inhibition of G0/G1 cell cycle arrest but not apoptosis³¹. Xiaojia Zhou also found metformin restrains the cell proliferation in SKM-1 cells through AMPK-regulated cell cycle arrest³². The study by Takuma Yamashita showed metformin prohibits the proliferation in gallbladder adenocarcinoma cell lines including NOZ, TGBC14TKB, and TGBC24TKB, and inhibits the cell cycle³³.

Besides the important biological processes, we also identified a number of key genes that involve in the treatment of liver cancer with metformin. Yueyue Yang et al found BRM2 is a liver cancer biomarker that indicates specifically increased levels in liver cancer and restrains ferroptosis by triggering GSH synthesis³⁴. The high expression of RRM2 was found in 81% of patients with liver cancer and the expression of RRM2 was associated with viral etiology and liver cirrhosis, which may be a biological marker for poor prognosis of liver cancer³⁵. CDC6 was identified as a therapeutic target of miR-215 in liver cancer³⁶. Moreover, CDC6 is an essential player for DNA replication, which is related to the risk for liver cancer³⁷. The circadian clocks and downstream targets are related to a variety of physiological and pathophysiological processes such as cell

proliferation, cell differentiation, cell metabolism, immune, and secretion³⁸⁻⁴⁷. Jorge Fung-Uceda et al found the clock component TOC1 can inhibit DNA replication by binding the CDC6 promoter⁴⁸. Bioinformatic analysis showed the CDC45 gene is closely associated with liver cancer and patient outcomes⁴⁹. Sheng-Ming Wu et al found the knockdown of UHRF1 can inhibit the progression of liver cancer⁵⁰. ASF1A is considered as a prognostic marker, which involves in the progression and development of specific cancers⁵¹. ZWINT plays key roles in the mitotic checkpoint, which can predict the pathogenesis and prognosis of liver cancer⁵². PCNA is identified as a potential marker for early hepatocellular carcinoma⁵³. ASPM is a biological marker for vascular invasion and poor prognosis of liver cancer⁵⁴. Kyu Yun Jang et al found SCTR1 and MYC drive liver tumor cell survival and predict liver cancer⁵⁵. Serological TK1 is a critical proliferation biomarker for the early discovery of pre-malignancies⁵⁶.

In conclusion, our study showed the significant impact of metformin in liver cancer. The DNA replication and cell cycle are the major signaling pathways involved in the treatment of liver cancer with metformin. Therefore, this study may be beneficial in the treatment of hepatocellular carcinoma.

Author Contributions

Tingting Zhang, Hongmei Guo, Letian Wang, Mengyao Wang: Methodology and Writing.
Hanming Gu: Conceptualization, Writing- Reviewing and Editing.

Funding

This work was not supported by any funding.

Declarations of interest

There is no conflict of interest to declare.

Figure 1. Heatmap and volcano plot were obtained between the control (CTRL) and metformin treated (TREAT) liver cancer cells

(A) Heatmap of significant DEGs. Significant DEGs ($P < 0.01$) were used to create the heatmap.

(B) Volcano plot for DEGs between the control and metformin treated liver cancer cells. The most significantly changed genes are highlighted by grey dots.

Figure 2. KEGG and GO analyses of DEGs between the control and metformin treated liver cancer cells

(A) KEGG analysis, (B) Biological processes, (C) Cellular components, (D) Molecular functions.

Figure 4. Reactome map representation of the significant biological processes between the control and metformin treated liver cancer cells

Table 1

Entrez gene	Gene symbol	Fold-change	Regulation
Top 10 down-regulated DEGs			
6236	RRAD	-5.641311351	Down
80201	HKDC1	-5.331256314	Down
4688	NCF2	-5.019051335	Down
343	AQP8	-4.538374629	Down
2331	FMOD	-4.360346053	Down
119395	CALHM3	-4.314311307	Down
9229	DLGAP1	-4.076112	Down
5649	RELN	-4.068611599	Down
153222	CREBRF	-3.688866916	Down
4495	MT1G	-3.639888941	Down
Top 10 up-regulated DEGs			
4973	OLR1	4.670169784	up
374393	FAM111B	4.38801419	up
4091	SMAD6	4.369002101	up
29128	UHRF1	4.290715153	up
6241	RRM2	3.73058798	up
9502	XAGE2	3.67535914	up
9415	FADS2	3.423215343	up
65263	PYCR3	3.392965269	up
54058	C21orf58	3.332096934	up
147011	PROCA1	3.285038179	up

Table 2. Top ten genes demonstrated by connectivity degree in the PPI network

Gene symbol	Gene title	Degree
RRM2	Ribonucleotide Reductase Regulatory Subunit M2	18
CDC6	Cell Division Cycle 6	17
CDC45	Cell Division Cycle 45	17
UHRF1	Ubiquitin Like With PHD And Ring Finger Domains 1	16
ASF1B	Anti-Silencing Function 1B Histone Chaperone	15
ZWINT	ZW10 Interacting Kinetochore Protein	15
PCNA	Proliferating Cell Nuclear Antigen	15
ASPM	Assembly Factor For Spindle Microtubules	14
MYC	MYC Proto-Oncogene, Bhlh Transcription Factor	13
TK1	Thymidine Kinase 1	12

References

- [1] Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR: A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019, 16:589-604.
- [2] Liu CY, Chen KF, Chen PJ: Treatment of Liver Cancer. *Cold Spring Harb Perspect Med* 2015, 5:a021535.
- [3] Lurje I, Czigany Z, Bednarsch J, Roderburg C, Isfort P, Neumann UP, Lurje G: Treatment Strategies for Hepatocellular Carcinoma (-) a Multidisciplinary Approach. *Int J Mol Sci* 2019, 20.
- [4] Irons BK, Minze MG: Drug treatment of type 2 diabetes mellitus in patients for whom metformin is contraindicated. *Diabetes Metab Syndr Obes* 2014, 7:15-24.
- [5] Lashen H: Role of metformin in the management of polycystic ovary syndrome. *Ther Adv Endocrinol Metab* 2010, 1:117-28.
- [6] Saraei P, Asadi I, Kakar MA, Moradi-Kor N: The beneficial effects of metformin on cancer prevention and therapy: a comprehensive review of recent advances. *Cancer Manag Res* 2019, 11:3295-313.
- [7] Ryu TY, Park J, Scherer PE: Hyperglycemia as a risk factor for cancer progression. *Diabetes Metab J* 2014, 38:330-6.
- [8] Hirsch HA, Iliopoulos D, Struhl K: Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proc Natl Acad Sci U S A* 2013, 110:972-7.
- [9] Verdura S, Cuyas E, Martin-Castillo B, Menendez JA: Metformin as an archetype immuno-metabolic adjuvant for cancer immunotherapy. *Oncoimmunology* 2019, 8:e1633235.
- [10] Gu H: Identification of biomarkers and pathways of mouse embryonic fibroblasts with the dysfunction of mitochondrial DNA. *bioRxiv* 2021:2021.04.05.438453.
- [11] Zhang M, Wang J, Gu H: Identification of biological processes and signaling pathways in the stretched nucleus pulposus cells. *bioRxiv* 2021:2021.11.23.469730.
- [12] Yuan G: Identification of biomarkers and pathways of mitochondria in sepsis patients. *bioRxiv* 2021:2021.03.29.437586.
- [13] Jing L, Letian W, Hanming G: Identification of driver genes and biological signaling for alcoholic myopathy. *Research Square* 2021.
- [14] Yu G, Wang LG, Han Y, He QY: clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012, 16:284-7.
- [15] Luengo A, Sullivan LB, Heiden MG: Understanding the complex-l-ty of metformin action: limiting mitochondrial respiration to improve cancer therapy. *BMC Biol* 2014, 12:82.
- [16] Luo X, Chen X, Wang L, Yang B, Cai S: Metformin Adjunct With Antineoplastic Agents for the Treatment of Lung Cancer: A Meta-Analysis of Randomized Controlled Trials and Observational Cohort Studies. *Front Pharmacol* 2021, 12:639016.
- [17] Kim SH, Kim SC, Ku JL: Metformin increases chemo-sensitivity via gene downregulation encoding DNA replication proteins in 5-Fu resistant colorectal cancer cells. *Oncotarget* 2017, 8:56546-57.
- [18] Kisfalvi K, Eibl G, Sinnott-Smith J, Rozengurt E: Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. *Cancer Res* 2009, 69:6539-45.
- [19] Yuan G, Huang Y, Yang S-t, Ng A, Yang S: RGS12 inhibits the progression and metastasis of multiple myeloma by driving M1 macrophage polarization and activation in the bone marrow microenvironment. *Cancer Commun*, n/a.
- [20] Fu C, Yuan G, Yang ST, Zhang D, Yang S: RGS12 Represses Oral Cancer via the Phosphorylation and SUMOylation of PTEN. *J Dent Res* 2020:22034520972095.
- [21] Yuan G, Yang S, Gautam M, Luo W, Yang S: Macrophage regulator of G-protein signaling 12 contributes to inflammatory pain hypersensitivity. *Ann Transl Med* 2021, 9:448.

- [22] Montero-Melendez T, Nagano A, Chelala C, Filer A, Buckley CD, Perretti M: Therapeutic senescence via GPCR activation in synovial fibroblasts facilitates resolution of arthritis. *Nat Commun* 2020, 11:745.
- [23] Yuan G, Yang S, Yang S: Macrophage RGS12 contributes to osteoarthritis pathogenesis through enhancing the ubiquitination. *Genes & Diseases* 2021.
- [24] Yuan G, Fu C, Yang ST, Yuh DY, Hajishengallis G, Yang S: RGS12 Drives Macrophage Activation and Osteoclastogenesis in Periodontitis. *J Dent Res* 2021:220345211045303.
- [25] Pavlos NJ, Friedman PA: GPCR Signaling and Trafficking: The Long and Short of It. *Trends Endocrinol Metab* 2017, 28:213-26.
- [26] Yuan G, Yang S, Yang S, Ng A, Oursler MJ: RGS12 is a critical proinflammatory factor in the pathogenesis of inflammatory arthritis via acting in Cox2-RGS12-NF kappa B pathway activation loop. *J Bone Miner Res: WILEY* 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, 2019. pp. 147-.
- [27] Yuan G, Yang S, Liu M, Yang S: RGS12 is required for the maintenance of mitochondrial function during skeletal development. *Cell Discov* 2020, 6:59.
- [28] Bohinc BN, Gesty-Palmer D: Arrestins in bone. *Prog Mol Biol Transl Sci* 2013, 118:335-58.
- [29] Yuan G, Yang S, Ng A, Fu C, Oursler MJ, Xing L, Yang S: RGS12 Is a Novel Critical NF-kappaB Activator in Inflammatory Arthritis. *iScience* 2020, 23:101172.
- [30] Sinnett-Smith J, Kisfalvi K, Kui R, Rozengurt E: Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: dependence on glucose concentration and role of AMPK. *Biochem Biophys Res Commun* 2013, 430:352-7.
- [31] Wang Y, Xu W, Yan Z, Zhao W, Mi J, Li J, Yan H: Metformin induces autophagy and G0/G1 phase cell cycle arrest in myeloma by targeting the AMPK/mTORC1 and mTORC2 pathways. *J Exp Clin Cancer Res* 2018, 37:63.
- [32] Zhou X, Kuang Y, Liang S, Wang L: Metformin inhibits cell proliferation in SKM-1 cells via AMPK-mediated cell cycle arrest. *J Pharmacol Sci* 2019, 141:146-52.
- [33] Yamashita T, Kato K, Fujihara S, Iwama H, Morishita A, Yamana H, Kobayashi K, Kamada H, Chiyo T, Kobara H, Tsutsui K, Okano K, Suzuki Y, Masaki T: Anti-diabetic drug metformin inhibits cell proliferation and tumor growth in gallbladder cancer via G0/G1 cell cycle arrest. *Anticancer Drugs* 2020, 31:231-40.
- [34] Yang Y, Lin J, Guo S, Xue X, Wang Y, Qiu S, Cui J, Ma L, Zhang X, Wang J: RRM2 protects against ferroptosis and is a tumor biomarker for liver cancer. *Cancer Cell Int* 2020, 20:587.
- [35] Lee B, Ha SY, Song DH, Lee HW, Cho SY, Park CK: High expression of ribonucleotide reductase subunit M2 correlates with poor prognosis of hepatocellular carcinoma. *Gut Liver* 2014, 8:662-8.
- [36] Xu H, Huang J, Hua S, Liang L, He X, Zhan M, Lu L, Chu J: Interactome analysis of gene expression profiles identifies CDC6 as a potential therapeutic target modified by miR-215-5p in hepatocellular carcinoma. *Int J Med Sci* 2020, 17:2926-40.
- [37] Xiong XD, Fang JH, Qiu FE, Zhao J, Cheng J, Yuan Y, Li SP, Zhuang SM: A novel functional polymorphism in the Cdc6 promoter is associated with the risk for hepatocellular carcinoma. *Mutat Res* 2008, 643:70-4.
- [38] Xu L, Cheng Q, Hua B, Cai T, Lin J, Yuan G, Yan Z, Li X, Sun N, Lu C, Qian R: Circadian gene Clock regulates mitochondrial morphology and functions by posttranscriptional way. *bioRxiv* 2018:365452.
- [39] Yuan G, Hua B, Cai T, Xu L, Li E, Huang Y, Sun N, Yan Z, Lu C, Qian R: Clock mediates liver senescence by controlling ER stress. *Aging* 2017, 9:2647-65.
- [40] Zhu Z, Hua B, Xu L, Yuan G, Li E, Li X, Sun N, Yan Z, Lu C, Qian R: CLOCK promotes 3T3-L1 cell proliferation via Wnt signaling. *IUBMB Life* 2016, 68:557-68.
- [41] Yuan G, Hua B, Yang Y, Xu L, Cai T, Sun N, Yan Z, Lu C, Qian R: The Circadian Gene Clock Regulates Bone Formation Via PDIA3. *J Bone Miner Res* 2017, 32:861-71.
- [42] Fan XF, Wang XR, Yuan GS, Wu DH, Hu LG, Xue F, Gong YS: [Effect of safflower injection on endoplasmic reticulum stress-induced apoptosts in rats with hypoxic pulmonary hypertension]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 2012, 28:561-7.

- [43] Zhu Z, Hua B, Shang Z, Yuan G, Xu L, Li E, Li X, Sun N, Yan Z, Qian R, Lu C: Altered Clock and Lipid Metabolism-Related Genes in Atherosclerotic Mice Kept with Abnormal Lighting Condition. *Biomed Res Int* 2016, 2016:5438589.
- [44] Yuan G, Xu L, Cai T, Hua B, Sun N, Yan Z, Lu C, Qian R: Clock mutant promotes osteoarthritis by inhibiting the acetylation of NFkappaB. *Osteoarthritis Cartilage* 2019, 27:922-31.
- [45] Mao SZ, Fan XF, Xue F, Chen R, Chen XY, Yuan GS, Hu LG, Liu SF, Gong YS: Intermedin modulates hypoxic pulmonary vascular remodeling by inhibiting pulmonary artery smooth muscle cell proliferation. *Pulm Pharmacol Ther* 2014, 27:1-9.
- [46] Zhu Z, Xu L, Cai T, Yuan G, Sun N, Lu C, Qian R: Clock represses preadipocytes adipogenesis via GILZ. *J Cell Physiol* 2018, 233:6028-40.
- [47] Cai T, Hua B, Luo D, Xu L, Cheng Q, Yuan G, Yan Z, Sun N, Hua L, Lu C: The circadian protein CLOCK regulates cell metabolism via the mitochondrial carrier SLC25A10. *Biochim Biophys Acta Mol Cell Res* 2019, 1866:1310-21.
- [48] Fung-Uceda J, Lee K, Seo PJ, Polyn S, De Veylder L, Mas P: The Circadian Clock Sets the Time of DNA Replication Licensing to Regulate Growth in Arabidopsis. *Dev Cell* 2018, 45:101-13 e4.
- [49] Lu HP, Du XF, Li JD, Huang SN, He RQ, Wu HY, Li MF, Wu WZ, Chen JT, Mo WJ, Chen G: Expression of Cell Division Cycle Protein 45 in Tissue Microarrays and the CDC45 Gene by Bioinformatics Analysis in Human Hepatocellular Carcinoma and Patient Outcomes. *Med Sci Monit* 2021, 27:e928800.
- [50] Wu SM, Cheng WL, Liao CJ, Chi HC, Lin YH, Tseng YH, Tsai CY, Chen CY, Lin SL, Chen WJ, Yeh YH, Huang CY, Chen MH, Yeh YC, Lin KH: Negative modulation of the epigenetic regulator, UHRF1, by thyroid hormone receptors suppresses liver cancer cell growth. *Int J Cancer* 2015, 137:37-49.
- [51] Hu X, Zhu H, Zhang X, He X, Xu X: Comprehensive analysis of pan-cancer reveals potential of ASF1B as a prognostic and immunological biomarker. *Cancer Med* 2021, 10:6897-916.
- [52] Zhu W, Zhang Q, Liu M, Yan M, Chu X, Li Y: Identification of DNA repair-related genes predicting pathogenesis and prognosis for liver cancer. *Cancer Cell Int* 2021, 21:81.
- [53] Ma S, Yang J, Li J, Song J: The clinical utility of the proliferating cell nuclear antigen expression in patients with hepatocellular carcinoma. *Tumour Biol* 2016, 37:7405-12.
- [54] Zhang H, Yang X, Zhu L, Li Z, Zuo P, Wang P, Feng J, Mi Y, Zhang C, Xu Y, Jin G, Zhang J, Ye H: ASPM promotes hepatocellular carcinoma progression by activating Wnt/beta-catenin signaling through antagonizing autophagy-mediated Dvl2 degradation. *FEBS Open Bio* 2021, 11:2784-99.
- [55] Jang KY, Noh SJ, Lehwald N, Tao GZ, Bellocin DI, Park HS, Moon WS, Felsher DW, Sylvester KG: SIRT1 and c-Myc promote liver tumor cell survival and predict poor survival of human hepatocellular carcinomas. *PLoS ONE* 2012, 7:e45119.
- [56] Wang Y, Jiang X, Wang S, Yu H, Zhang T, Xu S, Li W, He E, Skog S: Serological TK1 predict pre-cancer in routine health screenings of 56,178 people. *Cancer Biomark* 2018, 22:237-47.