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REVIEW

Interplay Between SIRT-3, Metabolism and Its Tumor Suppressor Role in Hepatocellular Carcinoma

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Abstract Sirtuins (SIRT), first described as nicotinamide adenine dinucleotide (NAD^+)-dependent type III histone deacetylases, are produced by cells to support in the defense against chronic stress conditions such as metabolic syndromes, neurodegeneration, and cancer. SIRT-3 is one of the most studied members of the mitochondrial sirtuins family. In particular, its involvement in metabolic diseases and its dual role in cancer have been described. In the present review, based on the evidence of SIRT-3 involvement in metabolic dysfunctions, we aimed to provide an insight into the multifaceted role of SIRT-3 in many solid and hematological tumors with a particular focus on hepatocellular carcinoma (HCC). SIRT-3 regulatory effect and involvement in metabolism dysfunctions may have strong implications in HCC development and treatment. Research literature widely reports the relationship between metabolic disorders and HCC development. This evidence suggests a putative bridge role of SIRT-3 between metabolic diseases and HCC. However, further studies are necessary to demonstrate such interconnection.

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Abbreviations

SIRT	Sirtuin
SOD2	Superoxide dismutase 2
HCC	Hepatocellular carcinoma
HBV	Hepatitis B virus
HCV	Hepatitis C virus
AIH	Autoimmune hepatitis
PBC	Primary biliary cholangitis
NASH	Nonalcoholic steatohepatitis
DM2	Type 2 diabetes mellitus
NAFLD	Nonalcoholic fatty liver disease
MnSOD	Superoxide dismutase 2
OSCC	Oral squamous cell carcinoma
NMNAT2	Nicotinamide mononucleotide adenylyltransferase 2
GC	Gastric cancer
MIAM	2-[1-(3-Methoxycarbonylmethyl-1H-indol-2-yl)-1-methyl-ethyl]-1H-indol-3-yl}-acetic acid methyl ester

Introduction

Sirtuins (SIRT) were first described as nicotinamide adenine dinucleotide (NAD^+)-dependent type III histone deacetylases [1]. In mammals, the sirtuin family is composed of seven members (SIRT-1–SIRT-7), which differ in tissue distribution, subcellular localization, enzymatic activity, and target proteins. SIRT-3 is the most studied member of the mitochondrial sirtuin family. It localizes mainly in the mitochondria, binding and deacetylating several enzymes involved in metabolism such as superoxide dismutase 2 (SOD2) and catalase [2]. The increased expression of these antioxidant proteins is related to SIRT-3 overexpression [3]. This protein is characterized by a conserved enzymatic core containing both the catalytic and NAD^+ binding domains [2].

A crucial aspect concerns the role of SIRT-3 in metabolism and cancer. Advancements of knowledge in this new field of research will allow for a better understanding of the mechanisms by which SIRT-3 can reprogram cellular metabolism and regulate different cancer processes.

Interestingly, growing evidence has proven a role for SIRT-3 in some important hallmarks of cancer (Fig. 1). SIRT-3 regulates cell survival, death, and metabolic pathways, maintaining the balance between health and disease [4].

Several studies have described an involvement of the enzyme in metabolic disorders [5–7].

The relationship between its role in metabolic diseases and in the development of hepatocellular carcinoma (HCC) could be of particular interest.

HCC is the sixth most frequent tumor in men and the third cause of cancer-related death worldwide. The majority of HCCs in humans are superimposed on chronic liver disease, as a result of hepatitis B and C virus (HBV and HCV) infection, excessive alcohol consumption, autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and nonalcoholic steatohepatitis (NASH) [8].

HCC incidence is significantly increasing in Western countries due to new metabolic-associated risk factors, including obesity, diabetes, and other causes of nonalcoholic fatty liver disease [9, 10].

This article reviews the experimental evidence of SIRT-3 involvement in metabolism and cancer, highlighting the interplay between metabolic dysfunctions and its tumor suppressor role in HCC.

SIRT-3 in the Regulation of Cell Metabolism

SIRT-3 is an important player in regulating mitochondrial functions such as metabolism and response to oxidative stress [11–17].

The function of SIRT-3 is connected to the metabolic state of the cell, since its enzymatic activity is controlled by the cellular NAD^+/NADH ratio. In this respect, NAD^+ serves as an activator, while NADH and nicotinamide as feedback inhibitors [18, 19].

Certain cellular stressors or a low energy state in the cell is responsible for NAD^+/NADH ratio increase, nicotinamide level decrease, and SIRT-3 activation [18, 19].

The SIRT-3 knockout mouse model has been used to clarify the physiological role of the enzyme in metabolism. Hebert et al. [20] described a loss of SIRT-3, using mass spectrometry during caloric restriction, suggesting that SIRT-3 is an important regulator in caloric restriction adaptation.

By contrast, an increased expression of the enzyme has been observed during dietary restriction [13, 15, 21]. For example in the study published by Hirschey et al., it has been reported that SIRT-3 knockout mice showed a reduced fat oxidation in different tissues, fatty liver and decrease in hepatic ATP production, glycaemia, and cold tolerance [21].

Metformin, a biguanide drug commonly used in patients with type 2 diabetes mellitus (DM2), was observed to have attenuated in vivo mitochondrial expression of SIRT-3 in primary hepatocytes: This was associated with an enhanced acetylation of several mitochondrial proteins, suggesting an involvement of metformin in the regulation of energy metabolism in liver and a potential therapeutic effect of this compound [22].

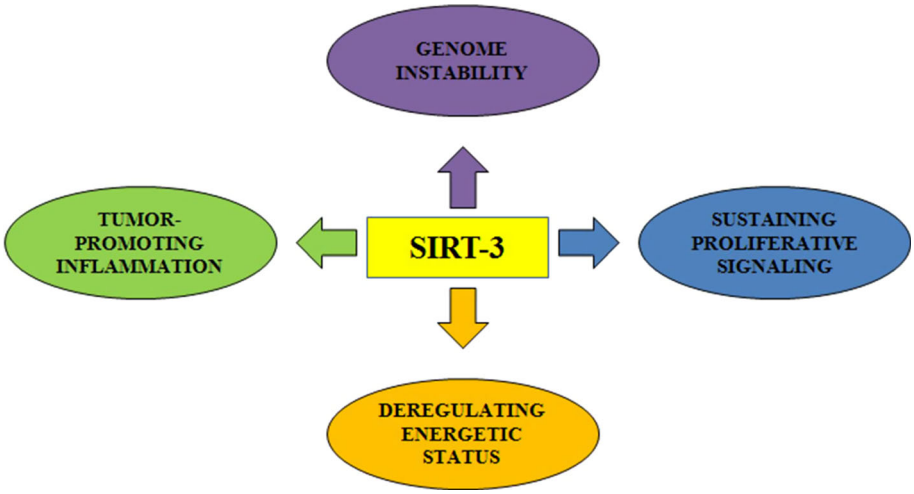

Fig. 1 SIRT-3 and the hallmarks of cancer. SIRT-3 can link to four hallmarks of cancer such as genomic instability, sustaining proliferative signaling, dysregulating energetic status, tumor-promoting inflammation

SIRT-3 and Metabolic Disorders

The above-mentioned studies suggest SIRT-3 as a promising therapeutic target against the dysfunctions that affect the mitochondrial metabolism [5–7].

Hirschey et al. [23] demonstrated how the reduction in SIRT-3 enzymatic activity and a high-fat diet feeding are associated with accelerated development of obesity, insulin resistance, and NASH. In this study, SIRT-3-deficient mice, under stress conditions, demonstrated that the lack of SIRT-3-mediated deacetylation of its target enzymes was insufficient for supporting the metabolic derangement caused by obesity and NASH. The authors also identified a functional single nucleotide polymorphism of the human SIRT-3 gene and associated with the metabolic syndrome development [23].

This study indicates that SIRT-3 plays a key role in protecting against mitochondrial oxidative stress through the regulation of the acetylation status of its target proteins.

The importance of SIRT-3 in regulating oxidative stress and impacting the pathogenesis of metabolic disorders such as NASH was also confirmed by He et al. [24] and demonstrated how manipulation of the expression or activity of SIRT-3 may represent a novel approach to manage NASH. According to this view, SIRT-3 could represent a potential therapeutic target in treating metabolic disorders.

In our opinion, this represents one important field of research to develop new therapeutic strategies for NASH management that, up to date, provides the lifestyle changes and an increased physical activity.

HCC and Metabolic Disorders

In the last years, DM2, metabolic syndrome, and NASH have been described as important risk factors for HCC development [25]. A 2.6% yearly incidence of HCC has been reported in patients with NASH-related cirrhosis [25].

Higher prevalence of obesity and diabetes mellitus has been related to increased nonalcoholic fatty liver disease (NAFLD), currently known as one of the causes of chronic liver disease worldwide [26, 27].

Considering the relationship between metabolic disorders and HCC development, the involvement of SIRT-3 in metabolic dysfunctions may have strong implications in HCC development and treatment. However, to date, there are no evidences on the interconnection among SIRT-3, metabolic disorders, and HCC. Further studies are necessary to demonstrate a putative bridge role of the enzyme.

Some retrospective studies and meta-analysis, performed on patients with DM2, suggested that metformin prevented HCC development [28–30], especially in those with chronic liver disease [31], while the use of sulfonylureas or insulin was associated with an increased risk of HCC [30]. Conversely, no significant association between thiazolidinediones use and HCC has been observed [30]. Based on meta-analysis results, diabetic patients at high risk for HCC development should be treated with metformin.

Dual Role of SIRT-3 in Cancer

The role of SIRT-3 in cancer is complex and controversial. Advancements of knowledge in this new field of research will allow for a better understanding of the mechanisms by which SIRT-3 can regulate different cancer processes.

The link among mitochondrial functions, metabolism, and cancer has been well reported in different solid tumors, where SIRT-3 regulates glycolytic and antioxidant genes expression, leading to a reduction in ROS production [17]. The scientific evidences about the role of SIRT-3 in cancer metabolism are reported in Table 1.

Tao et al. [32] observed a direct link between SIRT-3 and genomic stability. Indeed, SIRT-3 seems to regulate ROS production through manganese-containing superoxide dismutase 2 (MnSOD) and SOD modulation, promoting mutagenesis and genomic instability.

A study reported that SIRT-3 mediates metabolic reprogramming, destabilizing HIF-1α, and regulating glycolytic gene expression [33]. According to these observations [33], ROS production, glycolysis, and proliferation are inhibited by SIRT-3 overexpression, under hypoxic conditions, leading to HIF-1α stabilization and transcriptional activation [34, 35].

Different studies described a dual role of SIRT-3 in different solid and hematological tumors (Fig. 2).

In oral squamous cell carcinoma (OSCC), SIRT-3 resulted more expressed than normal oral tissues and its down-regulation inhibited OSCC cell growth and proliferation in vitro, indicating a tumor promoter role [36].

Lai et al. [37] nonetheless reported that levels of SIRT-3 and other sirtuins were significantly more down-regulated in tumor tissues than in normal tissues from patients affected by head and neck squamous cell carcinoma. Moreover, SIRT genes expression was shown to be more down-regulated in advanced than in early stages, causing the neoplastic disease to develop a more aggressive phenotype.

The pro-proliferative function of SIRT-3 has been also demonstrated in melanoma [38]. Indeed, SIRT-3 resulted overexpressed in a range of human melanoma cell lines with a different gene mutational status compared to normal primary melanocytes, suggesting the enzyme as a promising target for the treatment of this disease.

A recent study suggested that SIRT-3 plays a tumor-progressive role in renal carcinoma cells via glutamine-dependent oxidation, leading to reduced mitochondrial functions [39].

In breast cancer, the role of SIRT-3 remains contradictory.

Furthermore, Zhang et al. [40] showed that SIRT-3 expression levels increased in tamoxifen-resistant breast cancer cells and that SIRT-3 silencing sensitized these cells to tamoxifen, inducing apoptosis. On the contrary, another study described SIRT-3 as a tumor suppressor in human breast cancer, in part by regulating cellular metabolism through HIF-1α destabilization [33]. HIF-1α plays an important role in the Warburg effect in regulating several glycolytic genes and increasing anaerobic catabolism. This process supports the rapid proliferation of tumor cells. Finley et al. [33] demonstrated that a loss of SIRT-3 stabilized HIF-1α and increased glycolysis, whereas its overexpression repressed the Warburg effect in human breast cancer cell lines. This evidence suggests that the regulation of tumor cell metabolism by SIRT-3 could provide new ways of therapeutic approach.

Recently, it has been described a SIRT-3 up-regulation in human ovarian cancer cells after treatment with Bcl-2 inhibitors that interrupted glucose metabolism and induced apoptosis. This study suggests a tumor suppressor role of SIRT-3 in this solid tumor [41].

Low levels of SIRT-3 have been reported to induce energy metabolism-related apoptosis in non-small cell lung cancer cells through deacetylation of nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) and NAD + synthesis activity [42].

As shown by Xiao et al. [43], SIRT-3 expression appeared more down-regulated in human lung adenocarcinoma tissue than in the adjacent normal tissue. This study demonstrated that SIRT-3 overexpression-induced apoptosis-inducing factor (AIF) translocation to the nucleus up-regulated p53 and p21 protein levels and reduced intracellular ROS levels, exerting a pro-apoptotic function in this tumor.

Table 1 SIRT-3 in cancer metabolism

SIRT-3 functions	Key mechanism	Research team
Mutagenesis and genomic instability promoter	MnSOD and SOD modulation	Tao et al. [32]
Tumor suppressor	HIF-1α destabilization and glycolytic genes expression regulation	Finley et al. [33] Schumacker [34]
Tumor promoter	Glutamine-dependent oxidation	Choi et al. [39]
Tumor suppressor	Glucose metabolism interruption via Bcl-2 inhibition	Xiang et al. [41]
Tumor promoter	NMNAT2 deacetylation and NAD + synthesis activity regulation	Li et al. [42]
Tumor suppressor	ROS levels decrease via p53 and p21 up-regulation	Xiao et al. [43]
Tumor suppressor	ROS levels decrease and HIF-1α destabilization	Yang et al. [46]
Tumor suppressor	IDH2 and SOD deacetylation	Yu et al. [49]
Tumor suppressor	ROS levels decrease via SOD2 activation	Wang et al. [54]

SIRT-3 functions, key molecular mechanisms, and research team

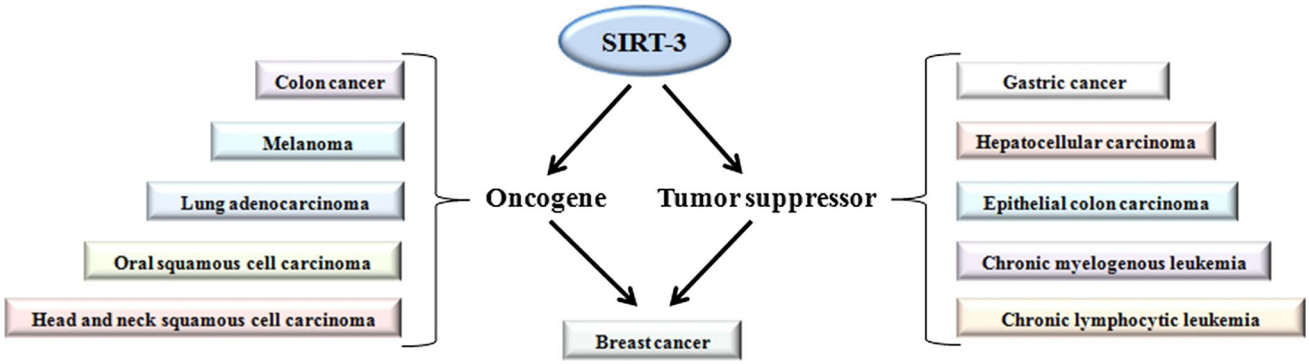

Fig. 2 Dual role of SIRT-3 in solid and hematological tumors

In line with this evidence, it has been reported that the enzyme promoted apoptosis, down-regulating Bcl-2 and JNK2 in HCT116 human epithelial colon carcinoma cells [44].

On the contrary, Liu et al. [45] demonstrated that a high SIRT-3 expression significantly correlated with high tumor grades, lymph nodes involvement, and poor prognosis in colon cancer patients.

Notably, SIRT-3 expression levels were inversely correlated with tumor infiltration and differentiation of gastric cancer (GC). SIRT-3 silencing could increase the expression of HIF-1α, suggesting its tumor suppressor role in GC [46].

In line with the results obtained by Yang et al. [46], it has been demonstrated that mRNA and protein levels of SIRT-3 were significantly reduced in human gastric cancer tissues and cell lines [47]. Moreover, Notch-1 overexpression inhibited the suppressor function of the enzyme on tumor cells proliferation, suggesting SIRT-3 as a novel therapeutic target in the gastric cancer treatment.

To date, few studies on the role of SIRT-3 in hematological malignancies have described the enzyme as tumor suppressor.

Marfe et al. [48] observed that the kaempferol treatment induced the inactivation of Akt signaling, the activation of caspase-3, followed by an increase in Bax and SIRT-3 and a decrease in Bcl-2 in chronic myelogenous leukemia K562 and promyelocytic human leukemia U937 cells.

Other findings have shown a SIRT-3 reduction in malignant B cell lines and primary samples obtained from chronic lymphocytic leukemia patients compared to controls from healthy donors. Decreased expression of the enzyme also correlated with hyperacetylation of IDH2 and SOD2 and higher ROS levels in these patients [49]. These results suggest that the activation of SIRT-3 pathway may represent a novel therapeutic approach for treating B cell malignancies.

As reported above, the role of SIRT-3 in cancer still remains ambiguous due to its possible function as an oncogene or a tumor suppressor, depending on the cancer cells and the pathways in which it is involved (Fig. 3).

The controversy regarding its dual role in cancer highlights the importance of investigating this research field. The knowledge of molecular mechanisms in several cancer types will clarify the oncogenic and tumor-suppressive function of SIRT-3 and help in developing a promising therapeutic strategy. Currently, in the early stages of clinical trials, SIRT-3 activators such as resveratrol have been tested for safety and cancer treatment [50].

The Tumor Suppressor Role of SIRT-3 in HCC

Mitochondria are the main site of ROS production, which has been reported as oncogenic factors and again are important site for apoptosis pathways. All of which seems to play a relevant role in hepatocarcinogenesis [51]. It has been demonstrated that ROS, produced by mitochondria, contributed to HCC progression and metastasis, inducing DNA damage or alteration of mitochondrial pathways [52, 53].

Several studies reported an involvement of SIRT-3 in tumor progression through HIF-1α and mitochondrial ROS production inhibition [33, 34].

SIRT-3 reduces ROS levels via SOD2, a major mitochondrial antioxidant enzyme that promotes oxidative stress resistance [4, 32].

Wang et al. [54] showed that the expression of SOD2 positively correlated with that of SIRT-3 in HCC cases, supporting the concept that SIRT-3 may reduce ROS production through the activation of SOD2. In agreement with the relationship between SOD2 and SIRT-3, several reports also showed a strong correlation between SOD2 reduced expression levels and HCC progression [55, 56]. Taken together, these data underlined the clinical relevance of SIRT-3 and its targets in HCC.

Several intracellular proteins are involved in the mitochondrial regulation of apoptosis, in particular the Bcl-2 protein family, which includes pro- and anti-apoptotic

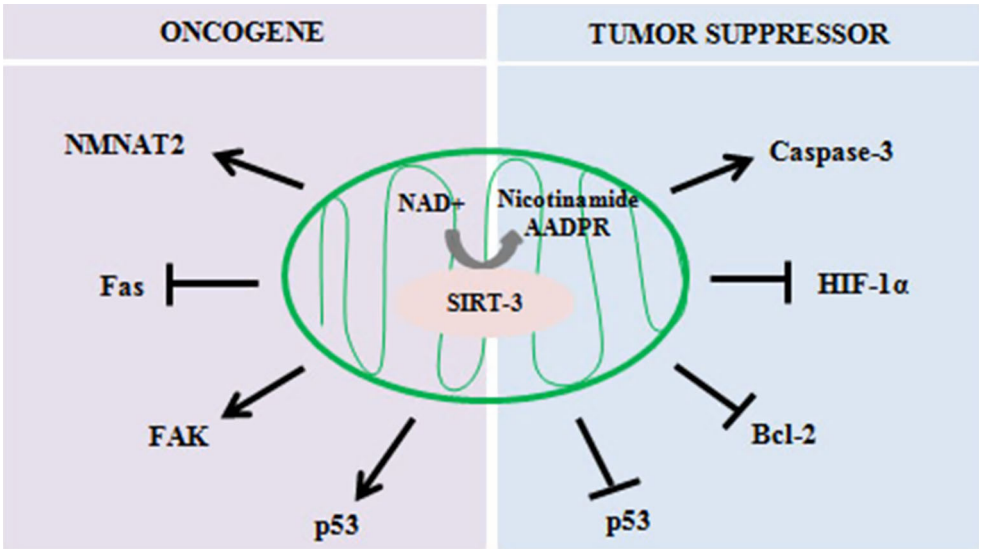

Fig. 3 SIRT-3 plays an either oncogenic or tumor-suppressive role in cancer, regulating several molecular effectors

members [57]. Among them, SIRT-3 is one of the main actors in the mitochondrial apoptotic pathway in HCC cells. SIRT-3 has been described as tumor suppressor in this neoplasm.

Song et al. [58] showed that GSK-3β localized in the mitochondria, where it interacted with SIRT-3, inducing Bax activation and translocation to the mitochondria. The same study revealed that overexpressed SIRT-3 up-regulated GSK-3β expression and activity, underlining its role as a tumor suppressor in HCC cells.

In line with that, mRNA and protein levels of SIRT-3 were lower in HCC tissue than in the peritumoral tissue and the reduced expression of the enzyme correlated with tumor de-differentiation, advanced staging, serum alpha-fetoprotein level, tumor multiplicity, and higher recurrence [59].

Moreover, Wang et al. [54] confirmed that SIRT-3 mRNA and protein levels were more down-regulated in HCC than normal livers and this reduction was significantly associated with tumor grade and size, highlighting the tumor suppressor role of the enzyme. The study also indicated that SIRT-3 may be considered a biomarker of recurrence after hepatectomy, in particular in BCLC stage A or no vascular invasion patient group.

It was found that the enzyme decreases hepatitis B virus x protein (HBx)-induced ROS production after viral infection [60]. This role in the regulation of oxidative stress after infection suggests that SIRT-3 could act in the control of fulminant hepatitis caused by viruses.

Furthermore, the overexpression of SIRT-3 repressed the extracellular signal-regulated kinase 1/2 pathway, partly blocked the activation of Akt, and down-regulated Mdm2, preventing p53 degradation with anti-tumor effect (Fig. 4) [61].

Even if SIRT-3 has been described as tumor suppressor in HCC, its role in drug sensitivity of liver cancer cells remains unclear.

Only a recent study highlighted a decreased SIRT-3 expression after the treatment with doxorubicin, cisplatin, and sorafenib in different HCC cell lines, suggesting a regulatory role of the enzyme in the drug sensitivity of HCC cells [62].

Another study demonstrated that fusaric acid-induced decreased mRNA and protein expression of SIRT-3 may be a putative mechanism of action in promoting cytotoxicity in HepG2 cell line [63].

Li et al. [64] demonstrated that HCC cells with high SIRT-3 expression levels were more sensitive to 2-[1-(3-Methoxycarbonylmethyl-1H-indol-2-yl)-1-methyl-ethyl]-1H-indol-3-yl}-acetic acid methyl ester (MIAM) than control cells, suggesting that MIAM inhibited HCC growth, due to SIRT-3 increase.

The results of another research reported that MIAM more strongly inhibited the growth of HCC in the resistant variants of Bel-7402 cell line through the activation of the NADPH oxidase 4 (NOX4)/p22, SIRT-3/SOD2 and SIRT-3/p53/p21 pathways [65]. This evidence suggests that MIAM could be developed as a potential treatment agent for HCC.

Conclusion

The role of SIRT-3 in cancer remains controversial. The advancements of the knowledge in this new research field will allow for a better understanding of the mechanisms by which SIRT-3 can regulate different cancer processes. Moreover, SIRT-3 has been recognized as an important regulator of mitochondrial integrity. Its regulatory effects

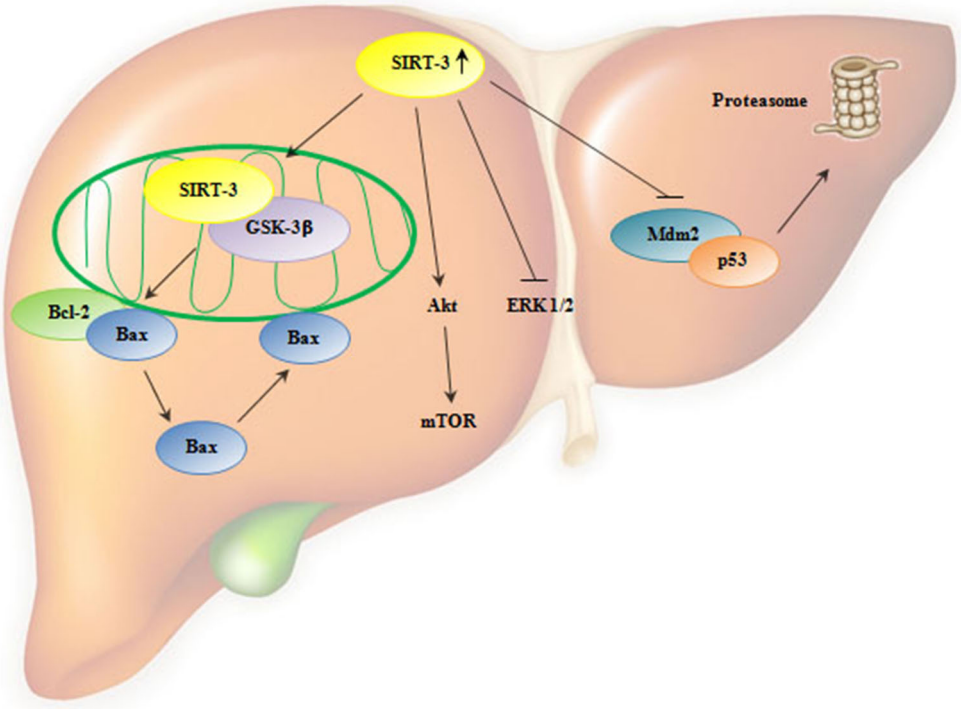

Fig. 4 The effect of SIRT-3 overexpression in HCC. SIRT-3 is involved in the regulation of GSK-3β/Bax, Akt/mTOR, ERK1/2, p53 signal pathways, promoting cell growth arrest and apoptosis in HCC cells

and involvement in metabolic diseases may have strong implications in HCC development and treatment. For this reason, the discovery of selective SIRT-3 activators could have an important role in giving new therapeutic alternatives. However, many questions on the role of SIRT-3 and its potential application in HCC remain unanswered, considering the complex background of this disease. We hypothesize that the effects of SIRT-3 activators could be different in HCC patients at distinct etiology. Up to date, there are no evidences on the interconnection among SIRT-3, metabolic liver disorders or viral infections, and HCC. Further studies are necessary to demonstrate a putative bridge role of the enzyme.

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Compliance with ethical standards

Conflict of interest The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

1. Guarente L. The many faces of sirtuins: sirtuins and the Warburg effect. *Nat Med*. 2014;20:24–25.
2. Chen Y, Fu LL, Wen X, et al. Sirtuin-3 (SIRT3), a therapeutic target with oncogenic and tumor-suppressive function in cancer. *Cell Death Dis*. 2014;5:e1047.
3. Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A, Gupta MP. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. *J Clin Invest*. 2009;119:2758–2771.
4. Alhazzazi TY, Kamarajan P, Verdin E, Kapila YL. Sirtuin-3 (SIRT3) and the hallmarks of cancer. *Genes Cancer*. 2013;4:164–171.
5. Cho EH. SIRT3 as a regulator of non-alcoholic fatty liver disease. *J Lifestyle Med*. 2014;4:80–85.
6. Teodoro JS, Duarte FV, Gomes AP, et al. Berberine reverts hepatic mitochondrial dysfunction in high-fat fed rats: a possible role for SirT3 activation. *Mitochondrion*. 2013;13:637–646.
7. Choudhury M, Jonscher KR, Friedman JE. Reduced mitochondrial function in obesity-associated fatty liver: SIRT3 takes on the fat. *Aging (Albany NY)*. 2011;3:175–178.
8. Stroffolini T, Trevisani F, Pinzello G, et al. Changing aetiological factors of hepatocellular carcinoma and their potential impact on the effectiveness of surveillance. *Dig Liver Dis*. 2011;43:875–880.
9. Faloppi L, Scartozzi M, Maccaroni E, et al. Evolving strategies for the treatment of hepatocellular carcinoma: from clinical-guided to molecularly-tailored therapeutic options. *Cancer Treat Rev*. 2011;37:169–177.
10. Bellissimo F, Pinzone MR, Cacopardo B, Nunnari G. Diagnostic and therapeutic management of hepatocellular carcinoma. *World J Gastroenterol*. 2015;21:12003–12021.
11. Weir HJ, Lane JD, Balthasar N. SIRT3: a central regulator of mitochondrial adaptation in health and disease. *Genes Cancer*. 2013;4:118–124.
12. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. *Nature*. 2000;408:255–262.
13. Dhillon RS, Denu JM. Using comparative biology to understand how aging affects mitochondrial metabolism. *Mol Cell Endocrinol*. 2016. doi:10.1016/j.mce.2016.12.020.
14. Kim HS, Patel K, Muldoon-Jacobs K, et al. SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of

mitochondrial integrity and metabolism during stress. *Cancer Cell*. 2010;17:41–52.
15. Shi T, Wang F, Stieren E, Tong Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J Biol Chem*. 2005;280:13560–13567.
16. Sack MN, Finkel T. Mitochondrial metabolism, sirtuins, and aging. *Cold Spring Harb Perspect Biol*. 2012;4:a013102. doi:10.1101/cshperspect.a013102.
17. Ansari A, Rahman MS, Saha SK, Saikot FK, Deep A, Kim KH. Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. *Aging Cell*. 2017;16:4–16.
18. Carafa V, Nebbioso A, Altucci L. Sirtuins and disease: the road ahead. *Front Pharmacol*. 2012;31:3–4.
19. Nogueiras R, Habegger KM, Chaudhary N, et al. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. *Physiol Rev*. 2012;92:1479–1514.
20. Hebert AS, Dittenhafer-Reed KE, Yu W, et al. Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. *Mol Cell*. 2013;49:186–199.
21. Hirschey M, Shimazu T, Goetzman E, et al. SIRT3 regulates mitochondrial fatty acid oxidation via reversible enzyme deacetylation. *Nature*. 2010;464:121–125.
22. Buler M, Aatsinki SM, Izzi V, Hakkola J. Metformin reduces hepatic expression of SIRT3, the mitochondrial deacetylase controlling energy metabolism. *PLoS ONE*. 2012;7:e49863.
23. Hirschey M, Shimazu T, Jing E, et al. SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol Cell*. 2011;44:177–190.
24. He J, Hu B, Shi X, et al. Activation of the aryl hydrocarbon receptor sensitizes mice to nonalcoholic steatohepatitis by deactivating mitochondrial sirtuin deacetylase Sirt3. *Mol Cell Biol*. 2013;33:2047–2055.
25. Souza MR, Diniz F, Medeiros-Filho JE, Araújo MS. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. *Arq Gastroenterol*. 2012;49:89–96.
26. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology*. 2010;51:1972–1978.
27. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol*. 2017;14:32–42.
28. Chen HP, Shieh JJ, Chang CC, et al. Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. *Gut*. 2013;62:606–615.
29. Chen TM, Lin CC, Huang PT, Wen CF. Metformin associated with lower mortality in diabetic patients with early stage hepatocellular carcinoma after radiofrequency ablation. *J Gastroenterol Hepatol*. 2011;26:858–865.
30. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Antidiabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. *Am J Gastroenterol*. 2013;108:881–891.
31. Donadon V, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. *Liver Int*. 2010;30:750–758.
32. Tao R, Coleman MC, Pennington JD, et al. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. *Mol Cell*. 2010;40:893–904.
33. Finley LW, Carracedo A, Lee J, et al. SIRT3 opposes reprogramming of cancer cell metabolism through HIF1α destabilization. *Cancer Cell*. 2011;19:416–442.
34. Schumacker PT. SIRT3 controls cancer metabolic reprogramming by regulating ROS and HIF. *Cancer Cell*. 2011;19:299–300.
35. Koyama T, Kume S, Koya D, et al. SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells. *Free Radic Bio Med*. 2011;51:1258–1267.
36. Alhazzazi TY, Kamarajan P, Joo N, et al. Sirtuin-3 (SIRT3), a novel potential therapeutic target for oral cancer. *Cancer*. 2011;117:1670–1678.
37. Lai CC, Lin PM, Lin SF, et al. Altered expression of SIRT gene family in head and neck squamous cell carcinoma. *Tumour Biol*. 2013;34:1847–1854.
38. George J, Nihal M, Singh CK, Zhong W, Liu X, Ahmad N. Pro-proliferative function of mitochondrial sirtuin deacetylase SIRT3 in human melanoma. *J Invest Dermatol*. 2016;136:809–818.
39. Choi J, Koh E, Lee YS, et al. Mitochondrial Sirt3 supports cell proliferation by regulating glutamine-dependent oxidation in renal cell carcinoma. *Biochem Biophys Res Commun*. 2016;474:547–553.
40. Zhang L, Ren X, Cheng Y, et al. Identification of Sirtuin 3, a mitochondrial protein deacetylase, as a new contributor to tamoxifen resistance in breast cancer cells. *Biochem Pharmacol*. 2013;86:726–733.
41. Xiang XY, Kang JS, Yang XC, et al. SIRT3 participates in glucose metabolism interruption and apoptosis induced by BH3 mimetic S1 in ovarian cancer cells. *Int J Oncol*. 2016;49:773–784.
42. Li H, Feng Z, Wu W, Li J, Zhang J, Xia T. SIRT3 regulates cell proliferation and apoptosis related to energy metabolism in non-small cell lung cancer cells through deacetylation of NMNAT2. *Int J Oncol*. 2013;43:1420–1430.
43. Xiao K, Jiang J, Wang W, et al. Sirt3 is a tumor suppressor in lung adenocarcinoma cells. *Oncol Rep*. 2013;30:1323–1328.
44. Allison SJ, Milner J. SIRT3 is pro-apoptotic and participates in distinct basal apoptotic pathways. *Cell Cycle*. 2007;6:2669–2677.
45. Liu C, Huang Z, Jiang H, Shi F. The sirtuin 3 expression profile is associated with pathological and clinical outcomes in colon cancer patients. *Biomed Res Int*. 2014;2014:871263.
46. Yang B, Fu X, Shao L, Ding Y, Zeng D. Aberrant expression of SIRT3 is conversely correlated with the progression and prognosis of human gastric cancer. *Biochem Biophys Res Commun*. 2014;443:156–160.
47. Wang L, Wang WY, Cao LP. SIRT3 inhibits cell proliferation in human gastric cancer through down-regulation of Notch-1. *Int J Clin Exp Med*. 2015;8:5263–5271.
48. Marfe G, Tafani M, Indelicato M, et al. Kaempferol induces apoptosis in two different cell lines via Akt inactivation, Bax and SIRT3 activation, and mitochondrial dysfunction. *J Cell Biochem*. 2009;106:643–650.
49. Yu W, Denu RA, Krautkramer KA, et al. Loss of SIRT3 provides growth advantage for B cell malignancies. *J Biol Chem*. 2016;291:3268–3279.
50. Signorelli P, Ghidoni R. Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. *J Nutr Biochem*. 2005;16:449–466.
51. Faccioruso A, Villani R, Bellanti F, Mitarotonda D, Vendemiale G, Serviddio G. Mitochondrial signaling and hepatocellular carcinoma: molecular mechanisms and therapeutic implications. *Curr Pharm Des*. 2016;22:2689–2696.
52. Yoon CY, Park MJ, Lee JS, et al. The histone deacetylase inhibitor trichostatin A synergistically resensitizes a cisplatin resistant human bladder cancer cell line. *J Urol*. 2001;185:1102–1111.
53. Royce SG, Dang W, Yuan G, et al. Effects of the histone deacetylase inhibitor, trichostatin A, in a chronic allergic airways disease model in mice. *Arch Immunol Ther Exp*. 2012;60:295–306.
54. Wang JX, Yi Y, Li YW, et al. Down-regulation of sirtuin 3 is associated with poor prognosis in hepatocellular carcinoma after resection. *BMC Cancer*. 2014;14:297.

55. Casaril M, Corso F, Bassi A, Nicoli N, Bellisola G, Corrocher R. Decreased activity of scavenger enzymes in human hepatocellular carcinoma, but not in liver metastases. *Int J Clin Lab Res*. 1994;24:94–97.
56. Liaw KY, Lee PH, Wu FC, Tsai JS, Lin-Shiau SY. Zinc, copper, and superoxide dismutase in hepatocellular carcinoma. *Am J Gastroenterol*. 1997;92:2260–2263.
57. Fabregat I, Roncero C, Fernández M. Survival and apoptosis: a dysregulated balance in liver cancer. *Liver Int*. 2007;27:155–162.
58. Song CL, Tang H, Ran LK, et al. Sirtuin 3 inhibits hepatocellular carcinoma growth through the glycogen synthase kinase-3β/BCL2-associated X protein-dependent apoptotic pathway. *Oncogene*. 2016;35:631–641.
59. Zhang CZ, Liu L, Cai M, et al. Low SIRT3 expression correlates with poor differentiation and unfavorable prognosis in primary hepatocellular carcinoma. *PLoS ONE*. 2012;7:e51703.
60. Ren JH, Chen X, Zhou L, et al. Protective role of Sirtuin3 (SIRT3) in oxidative stress mediated by hepatitis B virus X protein expression. *PLoS ONE*. 2016;11:e0150961.
61. Zhang YY, Zhou LM. Sirt3 inhibits hepatocellular carcinoma cell growth through reducing Mdm2-mediated p53 degradation. *Biochem Biophys Res Commun*. 2012;423:26–31.
62. Tao NN, Zhou HZ, Tang H, et al. Sirtuin 3 enhanced drug sensitivity of human hepatoma cells through glutathione S-transferase pi 1/JNK signaling pathway. *Oncotarget*. 2016. doi:10.18632/oncotarget.10319.
63. Abdul NA, Nagiah S, Chuturgoon AA. Fusaric acid induces mitochondrial stress in human hepatocellular carcinoma (HepG2) cells. *Toxicon*. 2016;119:336–344.
64. Li Y, Wang W, Xu X, Sun S, Xu X, Qu XJ. {2-[1-(3-Methoxy-carbonylmethyl-1H-indol-2-yl)-1-methyl-ethyl]-1H-indol-3-yl}-acetic acid methyl ester (MIAM) inhibited human hepatocellular carcinoma growth through upregulation of Sirtuin-3 (SIRT3). *Biomed Pharmacother*. 2015;69:125–132.
65. Li Y, Wang W, Xu X, Sun S, Xu X, Qu XJ. {2-[1-(3-Methoxy-carbonylmethyl-1H-indol-2-yl)-1-methyl-ethyl]-1H-indol-3-yl}-acetic acid methyl ester inhibited hepatocellular carcinoma growth in bel-7402 cells and its resistant variants by activation of NOX4 and SIRT3. *Biomed Res Int*. 2015;2015:491205.