Chapter

Impaired Autophagy and Exosomes Release by Long-Term mTOR Pathway Activation Promotes Hepatocellular Carcinoma Occurrence and Invasion

Qirong Wen, Qingfa Zeng and Ting Li

Abstract

Mammalian target of rapamycin (mTOR) is highly expressed in various types of hepatocellular carcinoma (HCC). Clinically, HCC cases without inflammation and cirrhosis are also increasingly common, especially in patients with nonalcoholic fatty liver disease, more and more patients develop HCC, which is only characterized by hepatic steatosis. However, the molecular mechanisms underlying the development of non-inflammatory HCC remain unclearly. Our previous study demonstrated that overactivation of mTOR pathway in the liver promotes de novo lipid synthesis and eventually spontaneous formation of non-inflammatory HCC. The continuous activation of mTOR pathway, on the one hand, promotes the de novo synthesis of lipids, resulting in the production of a large amount of lipid in the liver; on the other hand, it inhibits autophagy, resulting in the inability of lipid to be removed in time and accumulate in the liver. Accumulated lipid peroxidation eventually develops into HCC. In addition, the continuously activated mTOR pathway inhibited the release of exosomes by reducing the expression of Rab27A, and *in vitro* experiments confirmed that hepatoma cells after Rab27A knockout were more prone to invasion and metastasis. The reduced release of exosomes may impair intercellular communication, especially with immune cells, thereby making HCC more prone to invasion and metastasis with less inflammation.

Keywords: mTOR pathway, liver cancer, autophagy, exosomes, mouse model

1. Introduction

Cancer is a type of disease that seriously threatens human health. Among all cancer types, liver cancer is a very common gastrointestinal malignancy and one of the leading causes of cancer-related deaths [1–3]. Despite impressive advances in medicine over the past few decades, due to the occult nature of liver cancer, the uncertainty of its pathogenesis, the lack of effective treatments [4, 5], early diagnosis

of liver cancer, the survival rate, and prognosis are extremely poor, and the 5-year average survival rate is less than 10% [2, 3].

Rapamycin signaling pathway is abnormally up-regulated in about 50% of liver cancer patients [6]. The mTOR signaling pathway can not only regulate the metabolism of nutrients, such as nucleotides, lipids, and proteins but also inhibit autophagy and stimulate cell growth [7, 8]. Since the mTOR pathway plays a major role in a variety of metabolic and physiological processes, it also contributes to diseases and pathological conditions, such as aging, metabolic syndrome, and cancer when it is dysregulated [8]. As the global incidence of metabolic syndrome and obesity continues to rise, the accompanying liver cirrhosis and liver cancer are also increasing year by year, seriously threatening human life and health [9]. At the same time, there is also research evidence that lipid de novo synthesis plays a key role in the occurrence and development of human liver cancer, and data reveal that the mTOR pathway is the main regulatory pathway for abnormal lipid synthesis in liver cancer [10].

Our previous research report also found that chronic overactivation of mTOR pathway promotes de novo lipid synthesis in mice and eventually develops into hepatocellular carcinoma (HCC) [11]. Interestingly, the development of HCC in such mice was not accompanied by overt necroinflammation and liver fibrosis [12]. However, the pathogenesis of liver cancer is very complex, and the regulation of mTOR pathway is also very extensive. The involvement of mTOR pathway in regulating the occurrence and development of liver cancer may not be a single event but may involve the participation of multiple factors. Therefore, in this chapter, we will further explore the related mechanism of mTOR pathway involved in regulating the occurrence and development of liver cancer.

2. The mechanism of mTOR pathway involved in regulating the occurrence and development of liver cancer is very complex

2.1 Long-time chronic activation of mTOR pathway resulted in spontaneous HCC

2.1.1 HCC independent of long-term chronic injury and necroinflammation

The mTOR pathway is altered in various disease models and exhibits abnormal activation in tumor diseases, such as breast cancer, prostate cancer, lung cancer, liver cancer, kidney cancer, and lymphoma [13]. The TSC1/TSC2 heterodimer, consisting of tuberous sclerosis complex 1 (TSC1) and tuberous sclerosis complex 2 (TSC2), is an upstream inhibitor of mTOR pathway. Our previous data suggested that liver-specific knockout of TSC1 in mice leads to persistent activation of mTOR pathway that resulted in spontaneously HCC (**Figure 1A** and **B**). Histopathological features showed that the tumor cells were large with large nuclei and various staining, which was the pathological nuclear feature of malignant tumors. And only the cancer type of HCC was detected in these mouse models, indicating that the tumor was of hepatocyte origin and not of hepatic progenitor or bile duct epithelial origin (**Figure 1B**). Interestingly, these HCC model mice did not develop obvious inflammation and fibrosis, and the serum inflammatory factor levels did not increase significantly (**Table 1**), indicating that the continuous activation of mTOR pathway caused spontaneous HCC that was independent of long-term chronic injury and necroinflammation.

Studies have found that HCC is usually triggered by the death of liver cells, which usually leads to liver damage and secondary inflammatory response.

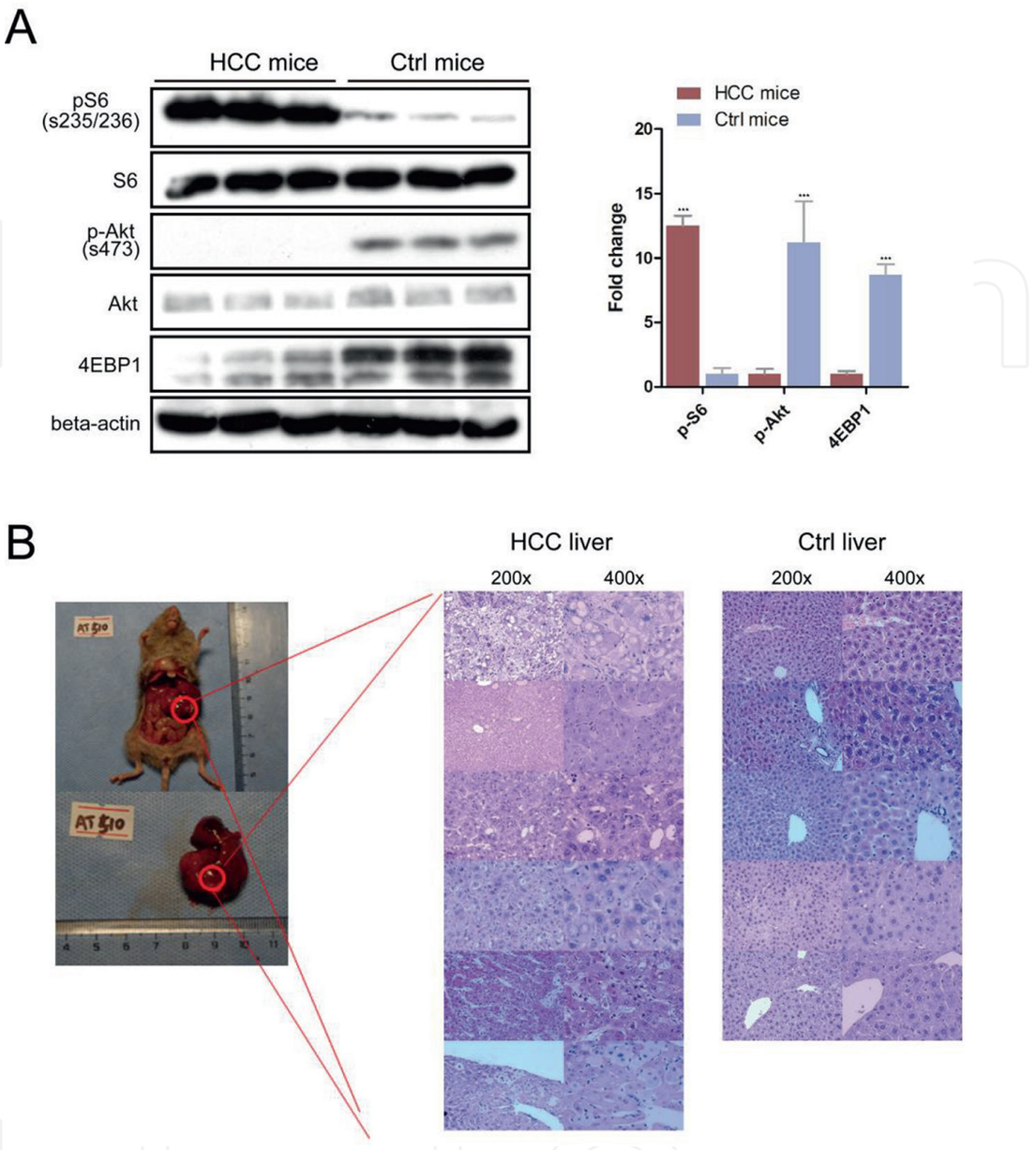

Figure 1.
*Persistent activation of mTOR pathway resulted in spontaneous HCC. (A) Western blots analysis showed the expression of mTOR pathway-related proteins in mouse liver tissue. Western blots were quantified based on at least 3 replicates. Error bars represent the SEM. ***P < 0.001. The resulting figure is cited from author's previously published article [11]. (B) the general picture of one spontaneous HCC mouse (left) and the pathological staining results of HCC mice and control mice (right), the magnifications are 200x and 400x.*

Therefore, majority of HCC tissues are accompanied by significant inflammation. Tumor inflammation is primarily caused by pro-tumor cytokines, including IL-6, that induces activation of the oncogenic transcription factor signal transducer and activator of transcription 3 (STAT3) in the hepatocytes, ultimately promoting compensatory proliferation of hepatocytes that have escaped cell death and subsequently promotes tumor development [14–16]. Activation of STAT3 further promotes IL-6 production and promotes inflammatory outbreaks. In a mouse model with liver-specific knockout of Raptor, a subunit of mTOR pathway, it was found that the increase of IL-6 and the activation of STAT3, ultimately promoted the occurrence and development of HCC, and showed mild liver cirrhosis in the process of developing

Factors	Ctrl mice (n = 5)	HCC mice (n = 9)	P-Value
IL-1a	52.87	49.76	0.6585
IL-1b	64.57	54.39	0.0904
IL-2	84.33	73.14	0.3589
IL-3	8.47	7.50	0.4328
IL-4	14.69	19.25	0.2418
IL-5	808.48	1083.47	0.4041
IL-6	319.35	283.91	0.5075
IL-9	8886.21	10211.62	0.1261
IL-10	32.61	31.45	0.7371
IL-12(p40)	9.21	13.04	0.2568
IL-12(p70)	128.93	146.81	0.2912
IL-13	3690.65	4507.50	0.2183
G-CSF	16.61	16.14	0.9514
GM-CSF	14.52	14.48	0.9923
IFN-g	5395.06	4226.12	0.1253
KC	186.65	189.51	0.9405
MCP-1	181.32	1260.69	0.2414
MIP-1a	42.22	50.83	0.6072
MIP-1b	367.00	340.20	0.2998
RANTES	838.56	810.17	0.7478
TNF-a	4568.78	3764.13	0.4491

Table 1.
The spectrum of inflammatory factors in the liver of mice.

into HCC [14]. In addition, STAT3 can also be activated by inhibiting signal transducer and activator of transcription 5 (STAT5), which promotes hepatocarcinogenesis through TGF-β [17, 18], and STAT5 also has a role in suppressing inflammation. However, no significant increase in inflammatory factors, including IL-6 and TNF-α was found in our mouse model. Meanwhile, accompanied by the activation of STAT5 phosphorylation and inhibition of STAT3 phosphorylation. Moreover, studies by others and our previous studies have demonstrated that mTOR pathway regulates phosphorylation of STAT3 and STAT5 [19]. Therefore, the spontaneous HCC model without necroinflammation might be developed through the activation of STAT5 with simultaneous inactivation of STAT3. Moreover, because there is no long-term necroinflammatory stimulus, the fibrosis of liver tissue is not very obvious.

2.1.2 The mTOR pathway regulates lipid de novo synthesis to mimic the progression of NAFLD to HCC

Since the first case of NAFLD-related HCC was reported in 1990 [20], there has been increasing evidence of an association between NAFLD and HCC, HCC is a common and lethal malignancy worldwide. Some risk factors (such as HBV, HCV, or alcoholism) are recognized in most cases of HCC, but there are also HCC that was

not caused by these risk factors, and the incidence of such HCC is as high as 15–50%. In developed countries, these "unexplained" HCCs are mainly attributed to NAFLD [21]. Cancer cells are characterized by a shift in cellular metabolism to adapt to cancer cell growth and provide more energy, which is conducive to the continued development of carcinogenesis. The mTOR signaling pathway is also an important metabolic regulatory pathway. The mTOR pathway is abnormally activated in the liver and other tissues of patients with obesity, mTOR activation promotes fatty synthesis acid by upregulating the transcription, synthesis and nuclear transfer of sterol regulatory element-binding protein-1 (SREBP1) [22].

This spontaneous HCC mouse model also exhibits the features of metabolic disorders. We used the pathway enrichment analysis method on mouse liver tissue RNA-seq data to enrich the related enriched functional groups regulating the mTOR pathway. And found that metabolism-related pathways ranked first in all enriched pathways (**Figure 2**). These metabolic pathways mainly, include chemical carcinogenesis-related pathways, steroid hormone biosynthesis pathways, linoleic acid metabolism pathways, and cytochrome P450 (CYP450) metabolic pathways in xenobiotics.

In addition, the results metabolomic assay of liver tissue showed that oleic acid (C18:1) increased and stearic acid (C18:0) decreased, which increased membrane fluidity, promoting metabolism and proliferation. Stearic acid has hepatoprotective and anti-inflammatory potential and can reduce fibrosis after cholestasis-induced liver injury [23]. The anticancer effects of liver-specific Pten-deficient female mice are at least partly due to a marked reduction in the ratio of oleic to stearic acid in the liver [24]. Accumulation of fatty acids may interfere with cell signaling pathways and promote tumorigenesis by altering gene transcriptional regulation, which may be the mechanism by which NAFLD, characterized by fat accumulation, eventually develops into liver cancer.

In our mouse model, after treatment with rapamycin, the abnormal lipid metabolism was reversed, and the occurrence of HCC was also effectively reversed, further proved that abnormal lipid metabolism is involved in the occurrence and development of liver cancer.

2.2 Autophagy is involved in the regulation of the occurrence and development of liver cancer

2.2.1 Autophagy impairment in persistent mTOR pathway activation mouse model

In addition to regulating metabolism, the mTOR pathway is also a classic autophagy regulatory pathway. When the mTOR pathway is activated, it mediates the

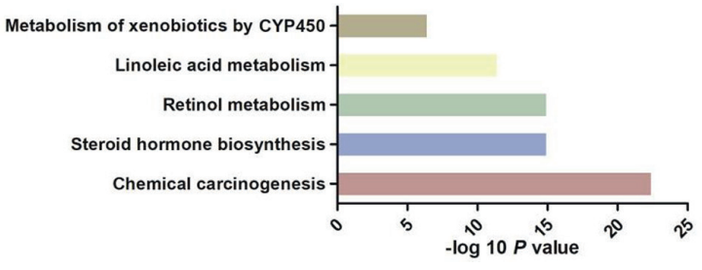

Figure 2.
Histographs of differentially expressed genes by pathway analysis.

phosphorylation of specific sites of ULK1 and Atg13 and inhibits the autophagy-promoting kinase activity of the ULK1 complex. When the mTOR pathway is inhibited, it is separated from ULK1, the phosphorylation of specific sites of ULK1 and Atg13 is released, the ULK1 complex is activated, and the activated ULK1 complex is then transferred to the isolation membrane of the endoplasmic reticulum, and autophagy is initiated [25].

Studies have found that AMPK/mTOR pathway-mediated autophagy activation is an important protective mechanism of NAFLD, mainly by inhibiting liver de novo adipogenesis, increasing fatty acid oxidation in the liver and promoting mitochondrial function/integrity in adipose tissue [26]. In the liver, autophagy, as a metabolic pathway, can regulate lipid accumulation in hepatic steatosis; while persistent mTOR pathway activation in hepatocytes leads to endoplasmic reticulum stress and autophagy defects, which are closely related to the occurrence and development of HCC. In our mouse model, sustained activation of hepatic mTOR pathway was found to lead to inhibition of autophagy. On the one hand, the continuous activation of mTOR pathway increases the de novo synthesis of lipids, and on the other hand, autophagy is inhibited, and the increased lipids cannot be efficiently metabolized and continuously accumulate in the liver. The study also believes that in the pathogenesis of NAFLD, autophagy is first activated and then inhibited, especially the continuous inhibition of autophagy will lead to the occurrence and further deterioration of NAFLD, which is consistent with our findings.

Furthermore, in the early stages of carcinogenesis, autophagy has significant cytoprotective and tumor suppressive potential. Dysfunction of this process is associated with an increased risk of cancer development. Therefore, we can also explain the phenomenon of spontaneous liver cancer in mice from the perspective of autophagy inhibition. The long-term continuous activation of the mTOR pathway keeps the autophagy in the liver of mice in a state of inhibition, and autophagy cannot effectively play the cytoprotective and tumor suppressive functions. Eventually, this leads to the occurrence of liver cancer. We demonstrated the above notion that rapamycin treatment in mice reversed autophagy impairment and prevented HCC development.

2.2.2 Activation of autophagy inhibits the invasion and metastasis of hepatoma cells in vitro

However, the role of autophagy in carcinogenesis has been controversial. Before tumorigenesis, effective autophagy can play cytoprotective and tumor suppressive effects, and autophagy can also be activated by tumor suppressor factors, such as PTEN, TSC, and DEPTOR [27, 28]. When tumors have already occurred, autophagy can further support tumor progression. Autophagy can promote tumor cell survival and malignant behavior by providing nutrients to tumor cells, thereby promoting tumor occurrence and development, while autophagy inhibition may make cancer cells. Sensitivity to metabolic stress conditions leads to tumor cell death [22, 29]. Many aggressive tumors require autophagy to facilitate important tumor-promoting processes [30].

However, some studies hold the different view. In a study by Zhang. et al., they found that SOCS5 promoted HCC cell migration and invasion *in vitro* by inactivating PI3K/Akt/mTOR pathway mediated autophagy. SOCS5 inhibition inhibited HCC cell migration and invasion *in vitro* by activating PI3K/Akt/mTOR pathway mediated autophagy. Dual inhibition of SOCS5 and mTOR further activates autophagy and exerts a more pronounced anti-metastatic effect in HCC cells [31].

In the spontaneous HCC mouse model, we found that on the basis of the continuous activation of the mTOR pathway and the inhibition of autophagy, some mice developed lung and intestinal metastasis of tumors (**Figure 3**). However, it was found that autophagy was activated and cell proliferation, invasion, and metastasis were decreased after treatment of liver cancer cells with rapamycin *in vitro* (**Figure 4A–D**). After the occurrence of tumors, autophagy may work with other mechanisms to maintain tumor homeostasis. When the effect of autophagy is relieved, it will destroy this homeostasis and promote tumor invasion and metastasis.

Future studies can pay more attention to the role of mTOR pathway mediated autophagy in different stages of liver cancer development, or explore other possible mechanisms to further clarify the mechanism of autophagy in different stages before and after tumorigenesis.

2.3 The occurrence and development of liver cancer involve complex mechanisms

The pathogenesis of tumors, including liver cancer, is very complex. In our spontaneous liver cancer mouse model, we found that in addition to abnormal STAT3/STAT5 pathway, lipid accumulation, and autophagy inhibition, there are many other abnormal manifestations.

2.3.1 Inhibition of exosomes secretion

It is well known that the mTOR pathway regulates autophagy to remove some cellular components, such as organelles to control cellular metabolism. The exosomes released and also transport part of the cell membrane and cellular components to the extracellular space, resulting in the loss of cellular contents. There is an overlap between autophagy and exosomes release, so the secretion of exosomes may also be regulated by the mTOR pathway. Our lab found that like autophagy, exosomes release is negatively regulated by the mTOR pathway in response to changes in nutrient and growth factor conditions, and the mTOR pathway mainly functions in the late stages of exosome biogenesis, possibly in the context of MVB (Multivesicularbody) and

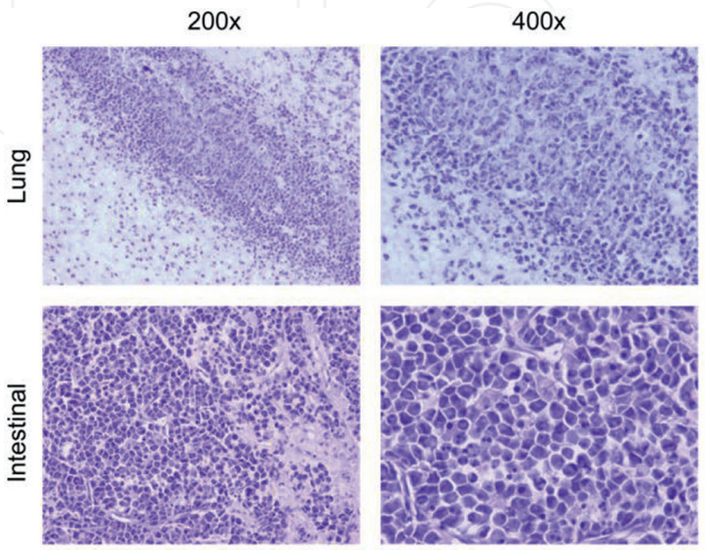

Figure 3.
The lung and intestinal metastasis in spontaneous HCC mouse. The magnifications are 200x and 400x.

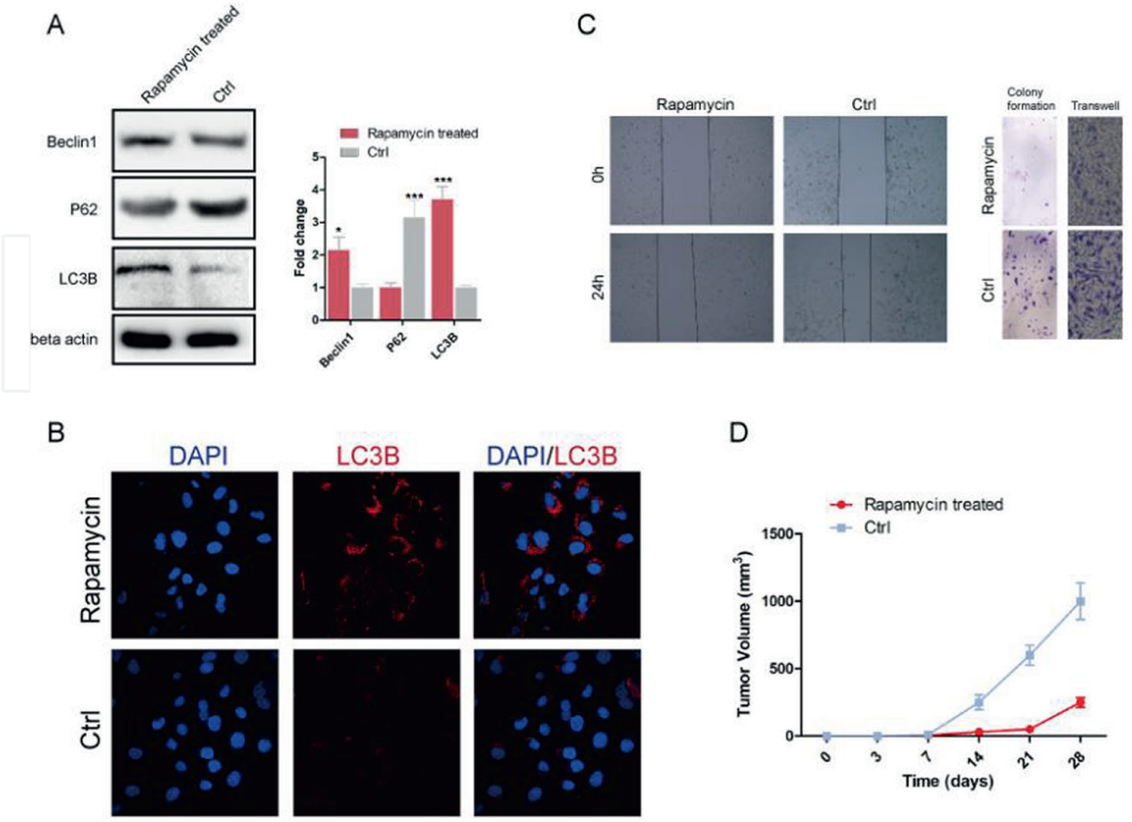

Figure 4.
*Autophagy activation inhibited tumor malignancy. (A) Western blots analysis showed the expression of autophagy-related proteins in Hep3B cells. Western blots were quantified based on at least 3 replicates. Error bars represent the SEM. ***P < 0.001. (B) Immunofluorescence analysis showed that rapamycin activates autophagy in Hep3B cells. (C) Wound healing experiments, clone formation, and transwell experiments confirmed that rapamycin treatment activates autophagy to inhibit the proliferation, invasion, and metastasis of Hep3B cells. (D) Tumor growth curves showed that autophagy activation inhibited tumor formation in nude mice.*

cytoplasmic membrane docking/fusion phase and validated in our mouse model, this inhibition was reversed after treatment with rapamycin [32]. We also detected a reduction of exosomes in the perisinusoidal space (Disse space) in this mouse model of spontaneous liver cancer in which knockout of TSC1 resulted in persistent mTOR pathway activation (**Figure 5A**). The inhibitory effect of mTOR pathway activation on the release of exosomes is mainly achieved by Rab27A, which does not change the content of exosomes. Rab27A and Rab27B are two small GTPases of the Rab family, which are involved in the docking and fusion of conditional MVB with the plasma membrane [33], which is crucial for the release of exosomes and the transmission of extracellular messengers, and it also regulates membrane homeostasis, lysosomal function, and autophagy.

The study found that cancer cells usually produce more exosomes than normal cells, exosomes from cancer cells have a strong ability to alter local and distant microenvironments, and exosomes from highly invasive tumor cells deal with low invasiveness tumor cells will enhance the latter's ability to invade and metastasize [34, 35]. In addition, tumor-derived exosomes can activate T cells, NK cells, or macrophages to activate immunity through direct or indirect antigen presentation [36]. Rab27A-overexpressing exosomes from tumor cells can further activate immunity, thereby promoting the proliferation of CD4+ T cells and exerting more effective anti-tumor immunity [37]. However, when Rab27A was silenced, Epithelial-mesenchymal

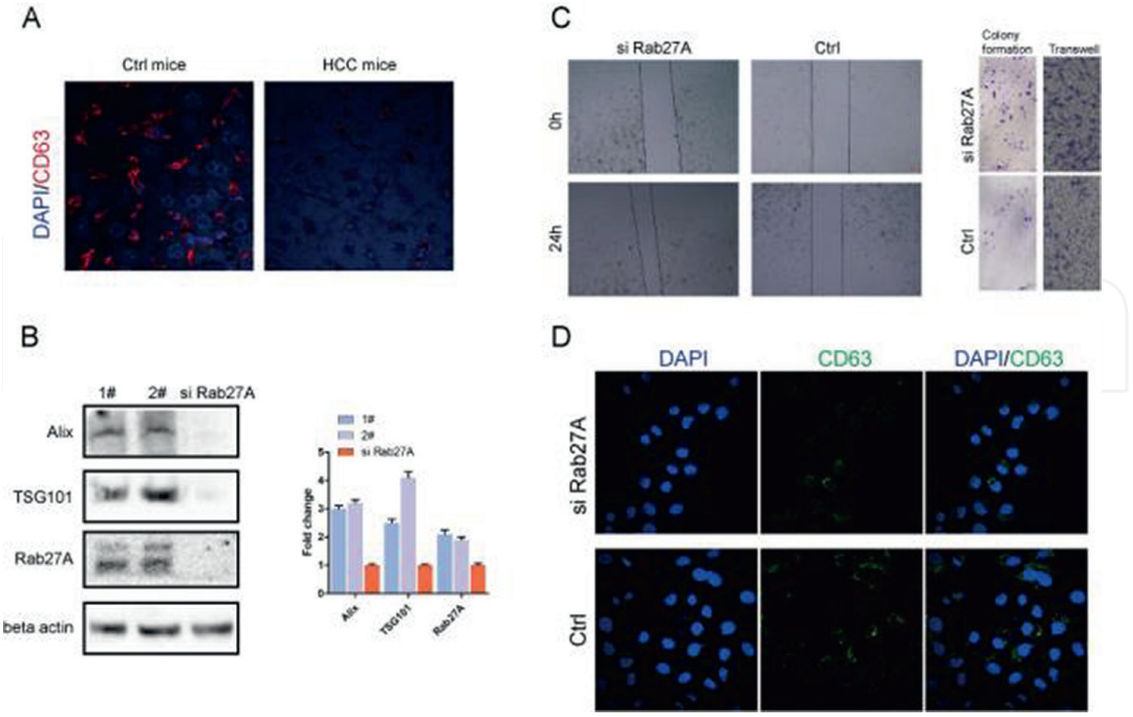

Figure 5.
Rab27A inhibited tumor malignancy. (A) Immunofluorescence analysis showed a reduction of exosomes in the Disse space in spontaneous HCC mouse model. (B) Western blots analysis showed the expression of Alix, TSG101, and Rab27A in 7721 cells. Western blots were quantified based on at least 3 replicates. (C) Wound healing experiments, clone formation, and transwell experiments confirmed that silencing Rab27A promoted the proliferation, invasion, and metastasis of 7721 cells. (D) Immunofluorescence analysis showed that silencing Rab27A inhibited the release of exosomes in 7721 cells.

transition (EMT) was induced through MAPK/ERK signaling pathway to promote cell migration, chemotaxis, and invasion, and the intrahepatic and lung metastasis increased [38]. Silencing the expression of Rab27A *in vitro* inhibited the release of exosomes and promoted the proliferation, migration, and invasion of tumor cells (**Figure 5B–D**). In our spontaneous liver cancer mouse model, the mTOR pathway was continuously activated, which continuously inhibits the release of exosomes, which may block intercellular communication from the perspective of exosomes, thereby failing to activate immunity and accelerate tumor development. The reason for the mild liver inflammation in this model mouse was also explained from the perspective of exosomes.

Recent studies have also shown the intersection of autophagy, exosome/amphoteric biogenesis, and exocytosis of extracellular vesicles [39, 40]. Autophagy is also involved in the exosome secretion process, changing not only the amount but also the content of exosomes. The effect of autophagy on the secretion of exosomes can be either promotion or inhibition, which may be closely related to the environment in which cells are located. This may partly explain the double-edged nature of autophagy in cancer progression.

2.3.2 Mitochondrial dysfunction

In 1956, Otto Warburg proposed that mitochondrial respiration defects were the potential basis of aerobic glycolysis and cancer [41], and the "Warburg effect" has been the basis of FDG-PET for tumor imaging. However, the mechanism of

mitochondrial action in cancer was unclear, although mutations in the mitochondrial genome have been identified in human cancer specimens [42]. Mitophagy is a specialized autophagy that selectively degrades and eliminates excess or damaged mitochondria [43]. Oncocytomas are rare benign tumors of most epithelial cells characterized by massive accumulation of defective mitochondria due to pathogenic mtDNA mutations [44]. In addition, the TCA cycle is also one of the metabolic pathways that occur within mitochondria, and how mutations in mitochondrial TCA cycle enzymes lead to cancer by producing oncogenic metabolites [45, 46].

Mitochondrial dysfunction and alterations in the TCA cycle were also found in our spontaneous liver cancer mouse model. It was mainly reflected indirectly by the decreased expression of Ddit4 and Nupr1. Among them, Ddit4 reflects mitochondrial function, and when its expression is abnormal, it disrupts energy homeostasis and promotes tumorigenesis, while functionally, the activation of mTOR pathway and cell survival requires the inhibition of Ddit4, and the inhibition of Ddit4 contributes to the continuous activation of mTOR pathway and tumorigenesis cell survival. Nupr1 is a mitochondrial-deficient gene involved in regulating autophagy induced by lipotoxicity of excess fatty acid accumulation in cells [47]. Nupr1 has also been identified as a key regulator and metabolic switch in response to mitochondrial damage during liver cancer development [48]. Changes in TCA cycle were manifested in the increased levels of fatty acid metabolites involved and the decreased levels of glucose metabolites involved. These changes all work together to promote the swearing development of liver cancer.

2.3.3 FGF21 alteration is a late event in spontaneous HCC

We also found a decrease in fibroblast growth factor 21 (FGF21) protein levels in the tumor tissue of the spontaneous liver cancer model mice, but there was no difference in FGF21 between model mice and control mice before tumorigenesis, and the reversal of FGF21 protein levels was not evident after treatment with rapamycin. Perhaps the change of FGF21 is only a concomitant phenomenon after tumorigenesis; however, FGF21 has been confirmed to promote the occurrence and development of NAFLD.

FGF21 is mainly expressed in liver, thymus, adipose tissue, and pancreatic islet beta cells [49], and it was regulated by the PI3K/AKT pathway to reduce blood sugar, reduce liver fat deposition, and reduce body weight [50]. Studies have shown that when the body was in a state of fasting and starvation, the expression of FGF21 in the liver and adipose tissue was induced, resulted in an increase in liver lipolysis and hepatic glycogen synthesis; an increase in adipose tissue glucose uptake, a decrease in lipolysis and an increase in lipogenesis [51]. FGF21 also reduces hepatic triglyceride and cholesterol production by inhibiting SREBP2 [52]. FGF21 was not only involved in the regulation of hepatic fat metabolism but also negatively regulated by the mTOR signaling pathway [53]. In addition, FGF21 may also be involved in the regulation of liver cell polarity and affect the normal structure of liver tissue (**Figure 6**). FGF21 regulated bile acid metabolism and also inhibited the expression of CYP7A1, which was the first step in the conversion of cholesterol to bile acids. Bile synthesis and secretion can promote the formation of hepatocyte polarity. When bile acid metabolism was disordered, hepatocyte polarity was also cannot maintained, and dysbiosis of bile acid metabolism was an important indicator for the pathological diagnosis of NAFLD. Deletion of FGF21 can affect both bile acid metabolism and lipid metabolism and ultimately promote the occurrence and development of NAFLD.

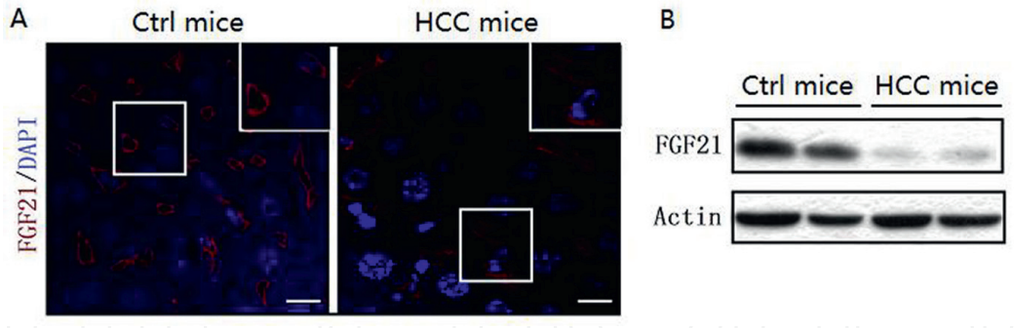

Figure 6.
Rab27A inhibited tumor malignancy. (A) Immunofluorescence analysis showed a reduction of FGF21 expression in spontaneous HCC mouse model. (B) Western blots analysis showed a reduction of FGF21 expression in spontaneous HCC mouse model.

In addition, FGF21 can aggravate tumor progression. Studies have found that reduced FGF21 protein levels are associated with cancerous hyperproliferation and abnormal p53, TGF-β/Smad signaling pathways during HCC development [54]; FGF21 can reduce hepatic fat accumulation and hepatocyte damage, while at the same time inhibiting inflammation and fibrosis and plays an important role in limiting progression of liver pathology from NAFLD/NASH to HCC [55, 56].

2.3.4 Ectopic expression of SLC22A7

Studies have found that SLC22A7 is highly expressed in liver cancer cells [57] and, SLC22A7 is associated with multicenter recurrence after liver cancer surgery, which may be achieved by regulating mitochondrial and oxidoreductase activities [58, 59]. In our spontaneous liver cancer mouse model, we found that SLC22A7 was not significantly altered at the protein level but was ectopically expressed. In normal liver, it was mainly expressed in the cell membrane of hepatocytes and in tumor liver, it was expressed in the cytoplasm (**Figure 7**).

2.3.5 Shows similar manifestations to chemical carcinogens

Further analysis of the results of transcriptome sequencing revealed that the spontaneous liver cancer mouse model has abnormal expression of many enzymes related genes, which are mainly involved in the detoxification process, including cytochrome P450 (CYP450) family, carboxylesterase (Ces), sulfotransferases (Sults), and UDP-glucuronyltransferases (Ugts). In the CYP450 family members, Cyp1a2, Cyp2b9, Cyp2c50, Cyp2c54, Cyp2c67, Cyp2e1, and Cyp3a16 expression decreased, while Cyp2b10 expression increased. CYP450 family-related enzymes are the key enzymes necessary for the phase I metabolism of exogenous substances in the liver. The Cyp450 family, such as Cyp1a2 and Cyp2e1 was a key enzyme in tumor transformation and mediates the metabolic activation of many carcinogens, which degrade xenobiotics, steroids, and fatty acids. Previous studies have found that the dysregulation of Cyp1a2 and Cyp2B9 mainly occurs in the liver of chemically carcinogenic mice [60, 61]. Another report found that Cyp2c50, Cyp2c54, and Cyp2c67 were significantly increased in the liver in a mouse model of chemically induced hepatocellular carcinoma [62]. Some researchers used Cyp2e1 knockout mice to study the effect of chemical carcinogenesis and found that Cyp2e1 knockout mice showed lower tumor incidence and diversity, indicating that the gene is a tumor protection related gene [63, 64].

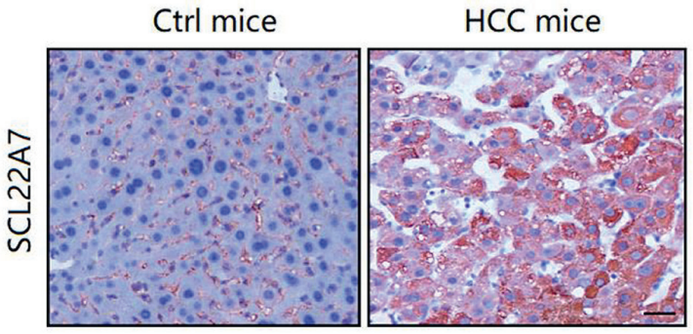

Figure 7.
Ectopic expression of SLC22A7. Immunohistochemical staining showed that SLC22A7 was mainly expressed in the cell membrane of hepatocytes, while wrong expressed to the cytoplasm in tumor cells of spontaneous HCC mouse model.

The first stage of tumorigenesis following mTOR pathway activation was analogous to chemical carcinogenesis, providing the primitive cells that both responsive to metabolic alterations and had a greater proliferative advantage over surrounding normal cells. Ultimately, these cells can be clonally expanded and transformed into cancer cells for immortal proliferation.

3. Conclusion

This chapter discusses some factors related to the mTOR pathway that may be involved in the occurrence and prognosis of HCC. There are multiple responsible changes in this spontaneous liver cancer model mouse, including accumulation of lipids, non-necrotizing inflammation, mitochondrial dysfunction, autophagy inhibition, exosome release inhibition, and protein ectopic expression. These changes may play a role before the occurrence of tumors, at the initial stage of tumors, or after the occurrence of tumors, and jointly promote the occurrence and development of liver cancer. Of course, the occurrence and development of HCC is a very complex process, and its related mechanism has always been a research hotspot, and new possible mechanisms will be reported over time.

Continued research is still needed to overcome difficult problems and ultimately be used for clinical treatment of liver cancer. Prevent the occurrence of liver cancer and reduce the recurrence of liver cancer after surgery. May the world be free from any cancer.

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