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Review Article

The role and behaviour of mitochondrial creatine kinase in hepatocellular carcinoma and its potential use as a tumor detecting biomarker for cancer patients

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ABSTRACT

Hepatocellular carcinoma (HCC) is responsible for approximately 75% of all liver cancer cases, which is the seventh most prevalent cancer and the second most common cause of cancer mortality worldwide. The prognosis of HCC depends on its stage and the severity of liver disease at the time of diagnosis, but there are still problems in detecting and treating HCC patients on time and effectively. Being unable to diagnose HCC patients at an early stage and ineffective therapies for HCC patients with advanced stages are associated with the disease's high mortality. Liver transplantation could be a treatment option If patients are diagnosed early, but unfortunately, most patients are diagnosed at an advanced stage where chemotherapy is necessary. Thus, an effective strategy for early detection of HCC is necessary since there was no effective chemotherapy for advanced HCC for a long time, and therapies such as the anti-angiogenesis pathway can only extend the median survival from 7.9 months to 10.7 months which is a step forward but not enough. In this article, we review the role of MtCK in HCC and its potential use as a marker to see if using it can be beneficial to patients.

Keywords: Hepatocellular carcinoma, Biomarker, Mitochondrial creatine kinase, Liver cancer

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the dominant global risk factors for hepatocellular carcinoma (HCC), but the prevalence of these factors will probably decrease because of the HBV vaccination and better treatment of HCV and HBV carriers. On the other hand, many experts predict that Non-alcoholic fatty liver disease (NAFLD), because of the obesity problem, will soon become the leading cause of HCC in the United States and other western countries.[1] Other factors such as excessive alcohol consumption, Diabetes, Obesity, Tobacco use, and genetic susceptibility can also play an essential role regarding HCC.[2] Gender differences also affect the prevalence of HCC. Men are more susceptible to HCC than women, with the ratio of 2.5-3 to 1 in the US, which can reach 6 to 1 elsewhere in the world.

Creatine kinase (CK) acts as a significant controller of cellular energy homeostasis. CK facilitates the reversible conversion of creatine into phosphocreatine, which builds a large pool of diffusing phosphocreatine for temporal and spatial buffering of ATP levels. Therefore, CK plays a significant role in tissues with high energy demands, such as muscles and the brain. Consequently, Mitochondrial isoenzymes of CK (MtCK) also play a vital role in the energetics of oxidative tissue. CK and MtCK dysfunction is associated with pathological conditions which change their function through mechanisms like up-regulating MtCK expression to compensate diminished energy state or directly blocking MtCK by oxidates and radical damage.[3]

Because of the growing incidence of HCC in many countries and the poor prognosis of HCC, the need for

early detection of HCC is crucial so cancer can be detected and treated at an early stage. Alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) are the markers currently used for HCC screening. However, DCP cannot provide enough sensitivity even if combined with AFP. On the other hand, liver ultrasound can provide higher sensitivity and specificity for early detection of HCC, but its accuracy depends on the operator.[3]

Overexpression of MtCK has been reported in patients with malignant liver tissue. Moreover, an increase in activity of serum MtCK was reported in several malignant tumors, including hepatic cancer, gastric cancer, and lung cancer.[3] Therefore, it is reasonable to consider MtCK as a potential biomarker for detecting HCC or other malignancies, which we aim to study in this article.

HEPATOCELLULAR CARCINOMA

Epidemiology

Currently, Asia and Africa have the highest occurrence rate globally, with Mongolia as the country with the highest occurrence rate at 93.7 per 100,000 and China with the highest number of cases.[4,5] However, between the years 1978 and 2012, a decrease in the occurrence rate of HCC was observed in a large number of Asian countries and Italy but the rate of occurrence in many European countries, Oceania. India and the Americas were increased, although in recent years, the increased rate has been mitigated in many countries.[2]

The prognosis for HCC patients has seen some improvements over the past 15 years, but the situation is still poor. In the US, 2-years survival for HCC is less than 50% and 5-year survival is only 10%. HCC occurrence rate in most populations is directly related to age until about 75 years old, although the median age of diagnosis usually is lower.[2,4] Men have a two to a four-fold higher chance of diagnosing HCC than women in most countries.[4] However, in some countries, the occurrence ratio between men and women can be significantly higher or lower (e.g., Uganda (M: F ratio=1.1), France (M: F ratio=5.0)).[4] Racial/ethnic differences can also be significant, as seen in Table 1.[2]

Table 1: In 2016, American Indians/Alaskan Natives in the US had the highest occurrence (11.4 per 100,000), followed by Hispanics (9.8), Asians/Pacific Islanders (9.1), non-Hispanic blacks (8.1), and non-Hispanic whites (4.6).

Race/ethnicity	Occurrence (per 100,000) in the US
American Indians/ Alaskan natives	11.4
Hispanics	9.8
Asians/Pacific islanders	9.1
Non-Hispanic blacks	8.1
Non-Hispanic whites	4.6

Treatment

Liver carcinogenesis is a multi-stage process, and the risk factors for different types of liver cancer are different.[5] One of the contributing factors to the increased incidence of HCC in some industrialized countries might be the association of HCC and non-alcoholic fatty liver disease. The poor prognosis of HCC makes treatments like surgical resection and radiofrequency ablation (RFA) only effective in the early stage of HCC. Moreover, almost 70% of the patients with HCC experience recurrence within five years of first occurrence.[6]

One of the most common chemotherapy agents for treating HCC is Sorafenib which blocks RAF kinase, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) receptors. Sorafenib has been shown to increase patients' life expectancy by at least 7-10 months. Drugs such as sunitinib, brivanib, and other angiogenic inhibitors are still being tested and developed.[1] Other drugs such as regorafenib used for secondary treatment after sorafenib and lenvatinib, which is another first-line drug for HCC, do not seem to be any more beneficial than sorafenib. However, better treatment options are still needed.[1] It is also important to mention that these therapies are associated with side effects and unsatisfactory effectiveness. Further studies about the pathogenesis of HCC are needed to develop more effective therapies to increase the survival of hospitalized HCC patients.[7]

MECHANISMS OF DEVELOPMENT AND PROGRESSION OF HCC

The development and progression of HCC are affected by several factors. These factors include chronic viral hepatitis, non-viral hepatitis, chronic alcohol consumption, some diseases (obesity and diabetes), and ingestion of toxins. The host's geographical location and underlying conditions could also determine the distribution of risk factors.[1]

Viral hepatitis

HBV and HCV could cause viral hepatitis that leads to cirrhosis and HCC. Numerous mechanisms could be involved in HBV-induced HCC pathogenesis, including host HBV-DNA integration, DNA methylation, oxidative stress, and HBx protein. The risk of developing HCC is determined by the level of HBV-DNA in liver cells.[8]

Non-viral hepatitis

Significant factors in the development and progression of HCC include diabetes mellitus, alcohol abuse, cardiovascular disease, hepatitis, obesity, dyslipidemia, and NAFLD.[1] Moreover, accumulation of iron in the liver is associated with fibrosis progression in patients and thus, serum ferritin could be a potential marker for the detection of tumors.[9] However, Due to the lack of a clear

relationship between intrahepatic iron and iron in the blood, clarifying the pathological role of ferritin in the poor prognosis of non-viral HCC is a difficult task.[1]

HCC and obesity

HCC is also associated with obesity as it disrupts metabolism and causes inflammation. Obesity is a factor in developing NAFLD, steatosis, NASH, liver fibrosis, cirrhosis, and HCC. A sedentary lifestyle may also have a role in this disease. Disorders of lipid metabolism and suppression of energy balance in the liver due to a sedentary lifestyle and obesity contribute to the association between type 2 diabetes and NAFLD. Several studies have shown that high BMI, waist circumference, and type II diabetes are associated with a higher risk of liver cancer.[1,10]

The role of alcohol

Alcoholic liver disease is one of the major causes of HCC and may double the risk of HCC. Liver inflammation and damage are not directly resulted from pure ethanol, but the side effects of alcohol catabolism like acetaldehyde accumulation and free radicals could lead to oxidative stress, cell death, apoptosis, necrosis, and necroptosis. Moreover, excessive alcohol consumption could affect the epigenetic mechanisms that have a role in tumor genesis and alter methylation and DNA acetylation.[11] Furthermore, excessive alcohol use is associated with HCC by metabolic disorders such as acetaldehyde accumulation, hypomethylation, antioxidant and retinoic acid deficiency associated with inflammation, oxidative stress, hypoxia, and genetic instability.[1]

Other mechanisms of progression to cirrhosis

Other potential risk factors include genetic predisposition to congenital disabilities, toxic exposure (aflatoxin or arsenic-contaminated food), and autoimmune liver disease. Several congenital anomalies, including hereditary tyrosinemia, Wilson's disease, alpha-1 antitrypsin deficiency, and hemochromatosis, have been shown to predispose patients to liver cirrhosis and HCC.

Currently, liver ultrasound every six months is a standard procedure for screening HCC in cirrhotic patients, but the effectiveness of HCC screening for people at low risk for HCC, such as patients with hepatitis B or those with non-alcoholic steatohepatitis without cirrhosis, is still unclear. Recently, in patients with HBV, clinical scoring systems such as PAGE B have been proposed to identify patients at risk for HCC, non-cirrhotic patients with HBV, or non-cirrhotic patients with non-alcoholic steatohepatitis at risk of developing HCC.[1]

CREATINE KINASE

Creatine Kinase (CK) isoenzymes produce ATP and creatine by catalyzing phosphate groups' reversible transfer from phosphocreatine to ADP. Various types of CKs can be found in the cytosol and mitochondria of cells. Two genes are responsible for encoding the two forms of mitochondrial CKs, sarcomeric MtCK (sMtCK), which is found in striated muscle of vertebrates and ubiquitous MtCK (uMtCK) that is co-expressed in a plethora of cells and tissues with high energy demand with cytosolic brain-type subunits like the brain, placenta, kidney, testis, sperm, and endothelial cells.[12,13] Therefore, uMtCK could be one of the major players in cellular energy homeostasis.[14,15]

An essential characteristic of malignant cells is a metabolic phenotype in which the primary source of ATP production shifts from oxidative phosphorylation (OXPHOS) to aerobic glycolysis.[16] This phenomenon is named the Warburg effect, after Otto Warburg. Warburg theorizes that despite the decrease in mitochondrial respiration in cancer cells, this shift is vital for survival.[14] Figure 1 shows a comparison between sources of ATP production.

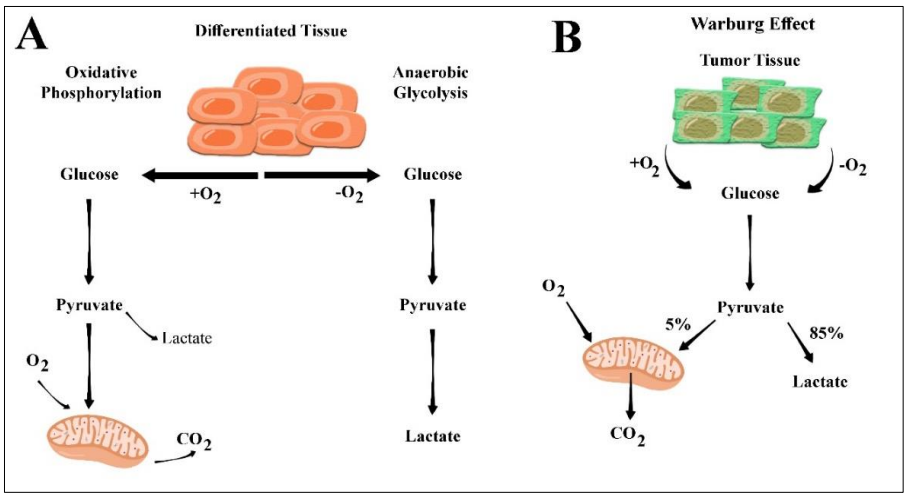

Figure 1: The shift from oxidative phosphorylation to aerobic glycolysis (Warburg effect), (A) the OXPHOS pathway results in production of 36 mol ATP/mol glucose. On the other hand, anaerobic pathway produces 2 mol ATP/mol glucose, (B) to aerobic glycolysis results in production of 4 mol ATP/mol glucose in tumor tissues.

The octameric mitochondrial and the dimeric cytosolic CKs are expressed in most vertebrate tissues. The mitochondrial isoenzyme diverged from the cytosolic isoenzyme about 670 million years ago, which indicates the need for specific functions but, on the other hand, the occurrence of the sarcomeric MtCK (sMtCK) and ubiquitous MtCK (uMtCK) is a relatively new phylogenetic event.[17] The sMtCK is found in vertebrates' striated muscle, and the uMtCK can be found in other tissues such as the brain, kidney, and sperm.[15]

The results from the immunogold electron microscopy showed that MtCK could be found in both the peripheral intermembrane space (IMS) and the cristae space.[18,19] the evidence from the rapid freezing techniques and electron microscopic tomography showed that IMS's small 9 nm height is only enough for MtCK octamer to fit between the two mitochondrial membranes.[20,21] The cristae space has typically enough room for MtCK octamers attached throughout the length of the cristae membrane as seen in Figure 2.[15]

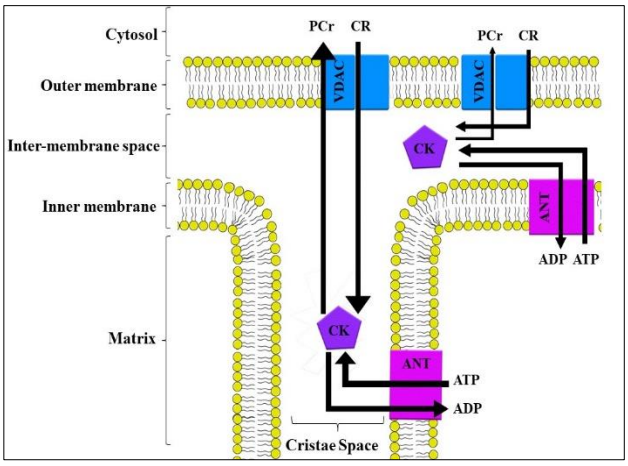

Figure 2: Localization of mitochondrial Creatine kinase. MtCK's has a direct interaction with VDAC which is regulated by Ca^{2+} while indirectly interacts with ANT. ANT: adenine nucleotide translocator, VDAC: Voltage-dependent anion channel.[15]

SUSCEPTIBLY OF CK TO REACTIVE SPECIES

MtCKs are the main targets of oxidative-induced molecular damages, and there are reports of their dysfunction in conditions such as ischemia, cardiomyopathy, and neurodegenerative disorders.[15,22] For example, in creatine-depleted muscles and patients with mitochondrial cytopathy, an upregulation of MtCK has been observed, which may have a compensatory role in mitigating the functional impairment of the energy state control. Moreover, there are reports of MtCK overexpression in tumors.[14,23,24]

Ischemia-reperfusion injury (IRI), along with other conditions such as neurodegenerative or age-related disorders, can cause mitochondrial dysfunction and produce reactive oxygen and nitrogen species (ROS, RNS).[25-28] The high susceptibility of CK to reactive species is highly related to the etiology of these conditions, which could result in interruption of the CK/phosphocreatine-shuttle and potentially impair cellular energetics as seen in double knockout mice without mitochondrial and cytosolic CK.[29] Apart from the high sensitivity of the MtCK, the reason it is a significant target of the ROS and RNS damage is its mitochondrial placement. Most of the reactive species result from the direct or indirect activity of the mitochondrial respiratory chain under conditions that increase oxidative stress.[15] Numerous in vitro and in vivo evidence has shown that ROS and RNS impede CK isoenzymes by enzymatic inactivation, but they also affect membrane binding capacity of MtCK and oligomeric state.[30-32] MtCK inactivation because of agents such as oxygen free radicals, NO, hydrogen peroxide, and superoxide are primarily irreversible, especially at higher concentrations of ROS, but they could also be partially reversible.[15] Under oxidative stress, the mitochondrial and cytosolic CK in muscles could be deactivated, which primarily results in impeded work performance and Ca^{2+} homeostasis. Regarding MtCK's function, this situation can damage oxidative tissues like the heart.[15,33]

MTCK'S COMPENSATORY BEHAVIOR AND UP-REGULATION

MtCK can be up-regulated in different health and disease conditions. During the development of skeletal muscle or even after endurance marathon training in human subjects or chronic electro-stimulation of muscle in animal subjects, adaptive changes in expression of CK isoenzyme could be observed, which results in a significant increase in the total amount of sMtCK, as well as its relative levels against MM-CK.[34,35] Another significant increase in MtCK activity is observed in the mitochondria of creatine-depleted animals fed with creatine analog β-guanidino propionic acid (β-GPA).[36] Up-regulation of MtCK has also been observed in the heart of rats stressed by chronic restrain, which expressed severe cardiac dysfunction, impaired mitochondrial respiration, and apoptotic cell death.[37] These examples suggest that sMtCK up-regulation compensates for improving oxidative energy metabolism. Moreover, the up-regulation of uMtCK is seen in some aggressive tumors, where MtCK could improve the low energy state, hypoxia, and nutrient restriction.[18,38]

Overexpression of uMtCK is observed in some tumors with poor prognosis.[23,24,38] Up-regulation of uMtCK is especially seen in Hodgkin's disease cell line.[39] Increased levels of uMtCK in malignant cells may help the cancer cells to proliferate in conditions with low oxygen and glucose.[40] An increase in MtCK levels could play a role in keeping the high energy turnover and protecting the cells from hypoxia or apoptosis. Moreover, transgenic liver expressing CK and supplemented showed protection against many deleterious metabolic insults like ischemia, hypoxia, or endotoxins.[41] Overexpression of uMtCK in

aggressive tumors may be explained by the ability to protect tumor cells from apoptosis.[15]

MTCK IN NERVOUS SYSTEM AND NEURONS

MtCK has a significant role in neurons, mainly because of the high energy consumption in the nervous system. Therefore, serious outcomes may occur if MtCK cannot function properly in the nervous system. It was reported that loss of uMtCK is associated with anomalous hippocampal mossy fiber connections, delayed seizure development, and other serious outcomes.[42,43] Other studies also indicated a decrease in the activity of MtCK in neurodegenerative diseases. A study showed that uMtCK activity is 63% lower in Huntington's disease human brain samples in comparison to non-diseased controls.[44]

In a study by Xu et al, a comparison between uMtCK, sMtCK, and CK-MB activity levels in serum of idiopathic Parkinson's Disease versus control group was made. The PD group showed a significant decrease in serum uMtCK activities. However, serum sMtCK and CK-MB activities showed no significant difference in the measured groups. Furthermore, the activity of serum uMtCK in different clinical dominant types of PD patients was measured, but no significant difference was found. Moreover, a direct correlation between serum uMtCK activity and rate of disease progression and age of onset was found. However, an inverse correlation between serum uMtCK and disease duration was also noticed.[42]

The decrease in uMtCK activity can be explained in several ways. It could adapt to both the decrease in mitochondrial function and the decline in energy supply seen in PD patients.[45] MtCK, which acts as an energy buffer system, may adapt to the new pathological state presented by PD and, therefore, reduce its expression as a similar phenomenon seen in HCC patients who show a higher activity of MtCK. Another scenario could be the reduced number of mitochondria because of mitochondrial damage, which leads to decreased uMtCK levels in peripheral blood.[42]

CK AS A BIOMARKER

A CK-MB-to-total-CK ratio of more than 1.0 can indicate conditions like cancer. Chang et al argue that a higher ratio of CK-MB-to-total-CK could be associated with some malignancies like hepatocellular carcinoma, lung cancer, and colorectal cancer or in conditions related to cancer such as liver metastasis. Based on this study, about 40% of patients with a CK-MB-to-total-CK ratio of more than 1.0 were diagnosed with malignancies such as colorectal cancer, lung cancer, and hepatocellular carcinoma. CK-MB-to-total-CK ratio is considerably higher in liver metastasis than in non-liver metastasis; also, the levels are higher in advanced stages of malignancies than in the early stages.[46]

There are reports of connections between increased mitochondrial CK activity and critical conditions like tumors.[47] The ratio between cancer stage and solid organ metastasis in advanced-stage cancer to CK-MB activity to CK-MB-to-total-CK ratio in patients with stage III/IV malignancies was significantly higher, but the estimated CK-MB activity had no statical difference between groups.[46]

Chang et al suggest that about 40% of patients with a CK-MB-to-total-CK ratio of more than 1.0 were diagnosed with malignancies. The most common of these malignancies are colorectal cancer, lung cancer, and hepatocellular carcinoma, respectively. The importance of using effective biomarkers for monitoring cancer is well known, but the high cost of using these biomarkers makes them unpopular. Therefore, using accessible biomarkers that can help with conditions like cancer is necessary.[46]

The increase in CK-BB activity was observed in malignancies such as lung, liver, breast, and prostate cancer. This increase could be because of an increase in enzymes due to tumor necrosis. The increase in CK-BB can be related to human liver metastasis.[46]

USING MTCK AS A MARKER FOR DETECTION OF CANCERS

Using MtCK as a marker has been suggested for years now. For example, Fusae et al reported an increase in MtCK levels in malignant tumors and suggested using MtCK as a marker.[24]

An increase in mitochondrial CK was seen in some cases of hepatocellular carcinoma. There were observations about reduced survival time in patients with liver cancer, suggesting that high MtCK expression can be related to poor prognosis in hepatocellular carcinoma.[3,48] Furthermore, there were indications that MtCK showed a cumulating risk for hepatocarcinogenesis in chronic hepatitis C patients.[49] There are also indications that overexpression of MtCK was linked to shorter overall survival time in breast cancer patients.[46,50]

Amamoto et al suggest that uMtCK is downregulated with prostate cancer progression. MtCK expression in high Gleason grade carcinoma was significantly lower than normal prostate or low-grade carcinoma. Furthermore, the Expression of uMtck in LNCaP and 22Rv1 cell lines and normal prostate cell line RWPE-1 was high in contrast to PC3 and DU145 and p53 cell lines, which almost had no expression. Moreover, in cell lines with low MtCK expression, an increase in glycolytic ATP production was observed while mitochondrial production was reduced. uMtCK increases in some cancerous tissues and may be involved in cancer progression because of its crucial role in OXPHOS and apoptosis in mitochondria. Therefore, uMtCK may be a novel marker for cancer.[14]

Qian et al. reported that uMtck expression is associated with poor prognosis in breast cancer and can be used as a tumor marker. Based on in vivo and in vitro evidence, uMtCK overexpression can help tumor growth by inhibiting apoptosis of tumor cells, stabilizing mitochondrial membrane potential, and reducing mitochondrial apoptotic pathway proteins. Thus, targeting the expression of uMtCK could be beneficial for breast cancer patients.[50]

INTERPLAY OF MIRNA AND MTCK AND ITS EFFECT ON HCC

Burchard et al suggested that miR-122, which regulates mitochondrial metabolism, is under-expressed in HCC. In addition, an increase in expression of miR-122 seed-matched genes leads to loss of mitochondrial metabolic function that could be detrimental to sustaining critical liver function. Therefore, miR-122 may play a significant role in the morbidity and mortality of liver cancer patients.[51]

MiR-122 is the most expressed miRNA in tumors and adjacent non-tumor tissues out of 220 miRNAs. miR-122 was decreased about three-fold in cirrhosis patients without HCC compared to non-tumor tissues in HCC patients. In addition, a 25% decrease was observed in tissues with hepatitis-B antigen relative to those without, which indicates that other liver diseases could also reduce mir-122 expression. However, the mechanism in which the decrease of miR-122 results in the repression of mitochondrially localized genes is not entirely understood yet.[51]

Burchard et al suggest that miR-122 may play a role in maintaining metabolic pathways associated with mitochondria and the normal function of mitochondria. Jopling et al argue that impaired mitochondrial functions, seen in many tumors, may not be related to miRNA but to the tumor itself, as seen in HCC.[52] In contrast, Burchard et al state that mitochondrial function pathways and miR-122 levels decline in the cirrhotic liver and are correlated. The increasing expression of miR-122 may potentially improve mitochondrial function in liver and liver tumor tissues.[51]

TARGETING MTCK BY MIR-519B-3P FOR INHIBITING THE DEVELOPMENT OF CANCER

In a study by Zhang et al it was suggested that uMtCK was the direct target of miR-519b-3p in colorectal cancer (CRC), and there was a negative correlation between them. Moreover, it was shown that miR-519b-3p suppressed the signaling pathway of uMtCK/Wnt in CRC cells.[44]

Expression of miR-519b-3p was significantly decreased in CRC tissues from which we may interpret its anti-tumor function in CRC cells. Overexpression of miR-519b-3p resulted in suppressing the proliferation of CRC cells, inhibition of CRC cell invasive ability, and lowered colony formation efficiency in CRC cells.

Overexpression of miR-519b-3p down-regulated the protein and messenger RNA levels of MtCK, and its inhibition resulted in increased expression of protein and mRNA levels of uMtCK. Up-regulation of mRNA expression of uMtCK was also observed in CRC tissues. Therefore, it can be concluded that uMtCK is a target gene of miR-519b-3p.

Zhang et al concluded that miR-519b-3p could be used in inhibiting the development of colorectal cancer by down-regulating uMtCK as uMtCK expression resulted in hindering the anti-tumor effect of miR-519b-3p.[44]

UTILIZING MTCK AS A BIOMARKER FOR DETECTION OF HCC

Expression of MtCK or cytosolic CK isoforms is assumed to be a sign of pathological development because, in general, the liver is one of the few tissues that does not express detectable levels of these enzymes.[3]

Soroida et al reported that serum MtCK was significantly increased in cirrhotic HCC patients. Moreover, serum MtCK activity in cirrhotic patients without HCC was also increased significantly compared to normal subjects, but the increase in cirrhotic patients with HCC was higher. The activity of the serum MtCK was not different between HBV and HCV patients, and no significant correlation between serum MtCK activity and BCLC stage was observed.[3]

Soroida et al also conducted an experiment to examine the potential predictability of serum MtCK activity, AFP, and DCP for differentiating HCC from cirrhosis without HCC. The results showed an increase in serum MtCK activity in more than half of HCC subjects. Moreover, the combination of AFP and MtCK led to an increase in sensitivity of HCC diagnosis. Similarly, the combination of DCP and MtCK also led to an enhanced diagnosis of HCC. However, diagnosing a number of patients failed even with utilizing MtCK, AFP, and DCP.[3] Table 2 shows a summary of the results of the experiment.

MtCK can also be used to support the result of ultrasonography diagnosis. Based on the study by Soroida et al, HCC was predictable in 84.7% of the ultrasound-detected HCC with the combination of MtCK and AFP, and 88.3% with the combination of MtCK, AFP, and DCP. Conversely, in the population of ultrasound-undetected HCC, HCC was predictable in 76.9% of patients with combination MtCK and AFP and 84.6% with the combination of MtCK, AFP, and DCP. Additionally, the activity of serum MtCK in about two-thirds of the patients with first HCC occurrence and HCC recurrence was higher than 8.0 U/l.[3]

The recommended screening strategy for cirrhosis patients includes measuring serum alpha-fetoprotein (AFP) levels and an abdominal ultrasound every six months for earlier detection of HCC. However, markers such as AFP, des-

gamma-carboxy prothrombin (DCP), and squamous cell carcinoma antigen-immunoglobulin M complex do not have optimal sensitivity and specificity unless used together.[53-55] Therefore, using MtCK as a marker might improve the condition of patients due to its increase in HCC (even in the early stage) and several other malignant tumors such as gastric cancer, breast cancer, and lung cancer.[23,24,50] However, in oral squamous cell carcinoma, uMtCK was down-regulated.[6]

Table 2: Number of patients with positive or negative results of each marker and their cut-off value.

Biomarker	Cut-off value	Diagnosis predicta-bility (%)	Sample size
DCP	40.0 mAU/ml	7	10
MtCK	8.0 U/l	14	21
AFP	20.0 ng/ml	6	9
AFP+DCP	-	9	13
DCP+MtCK	-	11	16
AFP+MtCK	-	23	34
MtCK+DCP +AFP	-	14	21
Triple negatives	-	16	23
Total	-	100	147

Ubiquitous MtCK was responsible for the majority of the increase in MtCK activity of HCC patients. After examination of other CK isoenzymes, no correlation between serum ubiquitous MtCK and other isoenzymes of serum CK activities was seen.[3]

A beneficial role for CK expression has been suggested even though its role in the pathological liver is not fully understood. CK expression in the liver of transgenic mice resulted in resistance against tumor necrosis factor-α-induced apoptosis, protection against hypoxia or endotoxin perfusion, and inhibition of pro-apoptotic mechanism.[3,56]

Uranbileg et al also suggested that patients with higher serum uMtCK activity had a poor prognosis, and the survival rate in patients with serum MtCK activity >19.4 U/l was shorter than in those with <19.4 U/l. Furthermore, serum MtCK activity was a significant risk factor for HCC-related mortality. In patients with serum MtCK >19.4 U/l, recurrence was observed earlier than those with MtCK <19.4 U/l. These findings suggest that HCC patients with higher serum MtCK may face a shorter survival time because of the higher malignancy potential.[6]

In HCC and cirrhosis patients caused by HCV and HBV virus, a significant increase in serum MtCK was observed. Moreover, serum MtCK was higher in cirrhosis patients with HCC in comparison to patients without HCC. MtCK in serum also decreased significantly after RFA treatment.[3]

Meffert et al reported that their evidence does not support the significant expression of CK in normal and pathologic liver, and they suggest that the other reports can be explained by interference from adenylate kinase (AdK) isoenzymes. They suggest that hepatoma cells were grown in tissue culture or increased levels of circulating BB-CK and MtCK in the blood of patients with liver tumors are responsible for inducing CK in liver tumors.[57,58] On the other hand, analysis of the tumor tissue itself, both by classical biochemical methods and microarray technology, did not show consistent results.[59-61] Moreover, mutations in the p53 tumor suppressor, which is quite prevalent in HCC, could directly or indirectly result in BB-CK induction in HCC because it was shown that p53 has a role in controlling BB-CK expression.[48]

In contrast to the earlier reports stating that the increase in serum MtCK was only detectable in advanced cases of HCC and that the sensitivity of serum MtCK for diagnosis of HCC was not high enough, in the study done by Soroida et al a relatively higher sensitivity of serum MtCK activity for detection of HCC was observed.[24,62] They suggest that using immune-inhibition instead of electrophoresis and densitometry used in previous studies led to enhanced results in quantifying MtCK activity.[24,62] As stated before, MtCK is found in dimeric and octameric forms, and after electrophoresis, dimeric MtCK is found near the position of CK-MM and thus, could result in overlapping of dimeric MtCK with CK-MM and causing miscalculation in the evaluation of MtCK activity.[3]

As opposed to previous studies, no correlation between serum MtCK activity and staging of HCC was observed. The releasing mechanism of MtCK into the bloodstream is not fully understood yet, and lack of correlation between the stage of HCC and MtCK releasing mechanism could explain this limitation.[3]

THE ROLE OF ASB9 IN REGULATION OF MTCK

ASB9 is a member of ASB protein families, which was found to interact with brain isoenzymes of creatine kinase and lead to its degradation. Reportedly, it was found that uMtCK is one of the ASB9 targets that induces its ubiquitination through the ankyrin domains of ASB9.[6,63]

In a study by Uranbileg et al, it was suggested that the increase in uMtCK level in HCC is not affected by mitochondrial integrity, but hepatocarcinogenesis by itself could be the contributing factor.[6,64]

Uranbileg et al studied the effects of ASB9 on uMtCK and HCC cells. Reportedly ASB9 plays a critical role in the regulation of uMtCK and brain type of creatine kinase. HCC cell lines like HEPG2, PLC/PRF/5, and HuH7 were selected to study the regulatory effect of ASB9 on uMtCK. ASB9 protein levels were almost undetectable in these cell lines compared to normal cells. Thus. Interaction between ASB9 and uMtCK may lead to degradation of uMtCK protein in HCC cell lines.[6]

Based on reports from Kwon et al, ASB9 negatively regulates uMtCK expression through inhibition of mitochondrial function, and therefore low uMtCK expression could be associated with loss of mitochondrial integrity.[65] However, Uranbileg et al reported that uMtCK expression was not changed in non-tumorous liver tissues with loss of mitochondrial integrity but showed an increase in HCC tissues. Thus, hepatocarcinogenesis is the interacting factor with uMtCK and not mitochondrial integrity. ASB9 reduces uMtCK levels in HCC cells, and uMtCK levels in the normal liver are usually at a low level while ASB9 mRNA expression is high, and therefore, ASB9 may play a physiological role to keep low uMtCK levels in the liver. Moreover, patients with colorectal cancer and low ASB9 expression had a higher chance of malignancy and poor prognosis in comparison to those with high ASB9 expression.[6]

Table 3: A review of the factors that affect MtCK and MtCK's reaction.

Condition/factor	Behavior of MtCK	Reference
Hodgkin's disease	Up-regulation of uMtCK is seen in the cell line	39
Huntington's disease	Decreased activity of 63% in brain samples of patients	66
Idiopathic Parkinson's disease	Decreased activity of uMtCK in contrast to sMtCK and CK-MB	42
Hepatocellular carcinoma	High expression of MtCK may associate with poor prognosis in patients	3, 48
Prostate cancer	Down-regulation might be associated with the progression of disease	14
Breast cancer	Overexpression of MtCK correlates to shorter survival time in patients	50
ASB9	negatively regulates uMtCK expression through inhibition of mitochondrial function	64
HCC and cirrhosis induced by HCV and HBV	Resulted in significant increase in serum MtCK	3
RFA treatment	MtCK in serum decreased significantly after the treatment	3

SUPPRESSION OF UMTCK USING SIRNA AND ITS EFFECTS

Uranbileg et al studied the effects of reducing the expression of uMtCK on Cell death, proliferation, migration, and invasion of HCC cells. SiRNA was used to suppress uMtCK expression in HepG2, PLC/PRF/5, and HuH7 cells and silence target protein expression. After 36 hours of transfection with uMtCK siRNA in HCC cell lines, uMtCK expression was significantly reduced. The reduction in uMtCK expression led to increased cell lysis, but no difference in caspase-3 activity was seen. Moreover, the reduction of uMtCK expression led to a decline in proliferation in all three HCC cell lines and, therefore, may have a role in keeping the active proliferation of HCC cells. Inhibiting the expression of uMtCK also resulted in decreased migration rate in the three HCC cell lines, and thus, uMtCK may have a role in the migration and invasion of HCC cells.[6]

MTCK IN HEPATITIS C AND LIVER FIBROSIS STAGING

In a study by Enooku et al, it was observed that the mean MtCK activity was increased in hepatitis C patients in comparison to the healthy subjects. Furthermore, unlike their previous studies, it was suggested that MtCK might be associated with the liver fibrosis stage. Moreover, a significant correlation between serum MtCK activity and serum levels of AST and ALT was seen to show a link between serum MtCK activity and hepatocellular damage. Serum AFP levels and serum MtCK activity also showed a significant correlation.[49]

Enooku et al also suggested that based on experiments on mice treated with bile duct ligation for four weeks, uMtCK mRNA levels in the liver were significantly increased compared to sham-operated mice. These results suggest that uMtCK expression, especially in hepatocytes, may increase in the fibrotic liver and potentially lead to increased serum MtCK activity. In conclusion, the higher serum MtCK activity in chronic hepatitis C patients may be an independent risk for HCC development.[49]

In general, healthy liver tissue does not express detectable levels of uMtCK and therefore could be a sign of pathological development; evidence from CK gene transgenic mice showed that CK expression in the liver could lead to inhibition of apoptosis and protect against hypoxia or endotoxin perfusion and thus play a protective role in injured liver tissue.[49] A review of the behavior of MtCK in relation to the conditions discussed can be found in Table 3.

CONCLUSION

The poor prognosis of HCC could be countered by using efficient and affordable biomarkers. Of course, no measure is as effective as preventing the condition in the first place with a healthy lifestyle and regular checkups. We can conclude that MtCK can be used as a complementary biomarker in HCC patients as it can increase the accuracy of diagnosis. However, using MtCK as a standalone marker does not seem like a reasonable choice as it can be

elevated or lowered due to various conditions in the body. Moreover, a better understanding of the relationship between the miRNAs and MtCK may lead to findings that could further improve the diagnosis and treatment of the disease. Ultimately, to further clarify the subject, we need more studies to find a practical use for MtCK in the diagnosis protocols.

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