Chapter

Selenium in the Prevention and Treatment of Hepatocellular Carcinoma: From Biomedical Investigation to Clinical Application

Chien-Shan Cheng, Ning Wang and Yibin Feng

Abstract

Selenium is a micronutrient that had been suggested to reduce the risk of cancer. Hepatocellular carcinoma (HCC), a prevalent disease and one of the most lethal cancers in the world, awaits new alternative treatment strategies to improve patients' survival. As an essential trace element, selenium has been studied for its anticancer properties in both oxidative stress and inflammatory-related mechanisms that may contribute to HCC growth and metastasis. In recent decades, increasing studies have investigated the potential role of selenium in liver cancer involving several major cancer-associated signaling pathways, metabolic pathways, and antioxidant defense systems both *in vitro* and in preclinical models. It was also observed that there was an increase in the trend of development of novel selenium nanoparticles and selenium-containing inhibitors aiming to improve the therapeutic efficacy and relative potency of selenium. However, controversies remain with whether a relationship exists between serum selenium level and HCC risk. This chapter aims to summarize the multi-target and multi-pathway *in vitro* and *in vivo* pharmacological effects of selenium in HCC, to provide a more comprehensive view and to highlight the recently discovered molecular mechanisms We hope this chapter could outline the correlation of selenium level and the risk of HCC in patients and discuss the clinical application of selenium in HCC prevention and treatment.

Keywords: selenium, hepatocellular carcinoma, anti-cancer, carcinogenesis

1. Introduction

Selenium (Se) is a naturally occurring essential micronutrient which had been received considerable attention in medicine and biology. As an essential component for mammalian cellular function, for the synthesis of several antioxidant enzymes, such as glutathione peroxidase, and for the synthesis of selenoproteins, selenium not only plays a vital role in balancing the redox environment in the body, but also is related to the prevention of diseases related to free radical damage, including infectious diseases, cancer, and cardiovascular diseases [1, 2]. The relationship between selenium and health has been the focus of medical community [3]. While

early observational studies have shown that the trace element selenium in the environment is closely related to the occurrence and development of tumors [3]. The incidence of cancer in patients with selenium deficiency is significantly increased, and the amount of selenium in the body is negatively correlated with cancer [4]. Furthermore, while some studies suggested selenium supplementation reduces the risk of cancer, some methodologically sound trials suggested selenium supplementation does not reduce the risk of cancer and may even increase it for some types, including advanced prostate cancer and skin cancer [5, 6]. An increased risk of diabetes has also been reported [7]. The relationship between selenium and cancer has been one of the most heated debates in human health over the past few decades. Moreover, the potential effect of selenium in the prevention and treatment of HCC is worthy of further investigation.

Primary liver cancer (PLC) is one of the most prevalent malignancies worldwide, and its incidence ranks fourth among all malignancies in China [8, 9]. The overall prognosis for patients with PLC is unsatisfactory with a 5-year survival rate of about 18%, and its mortality rate ranks the third in tumor-related death [10]. HCC is the most prevalent pathological type of PLC, accounting for about 85–90% of all PLCs [11]. Among all HCC patients, approximately 70–90% of them presented with a history of chronic liver diseases and cirrhosis [12]. While the primary risk factors for developing cirrhosis include chronic hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease, and nonalcoholic steatohepatitis (NASH), some other risk factors for HCC include uptake of aflatoxin-contaminated food, diabetes, obesity, certain genetic diseases such as hemochromatosis and some metabolic disorders [9, 11, 13]. Also, geographical factors have a direct impact on the various etiological features of HCC patients and make HCC an extremely complex condition associated with poor prognosis.

Epidemiology studies have found that the geographical factors and specific environmental exposures may be associated with HCC [14]. Inconsistent with the world's selenium distribution patterns, previous epidemiological studies have highlighted the particular relevance of selenium-associated cancer risk to the region of low selenium distribution, such as Asia or Africa [5, 15]. Some earlier surveying studies found that low environmental selenium is associated with certain cancers in the digestive system, and selenium supplementation may provide some cancer prevention effect [16]. However, the results had been inconsistent throughout various studies. Previous intervention trials in regions with low environmental selenium have shown a beneficial effect of selenium supplementation for cancer patients, while in most parts of North America, where there is sufficient environmental selenium, supplemental intake of selenium appears to be unrelated to chemoprevention [5, 17].

In Qidong, Jiangsu province, China, there is a high incidence of HCC and low environmental selenium level [18]. Various studies carried out in the region unraveled the negative correlation of HCC mortality with serum selenium level and environmental selenium level [19, 20]. However, in other areas in the world with low environmental selenium level, such correlation was not observed, suggesting that selenium deficiency may not be the sole cause of liver cancer and other factors, such as environmental carcinogens, the of heavy industry, chemical industry, etc. may collaboratively contribute to the high incidence of HCC [4].

Since the 1970s, continuous efforts had been made on studying the potential anti-cancer effect of selenium supplementation. Decrease of serum selenium level has also been observed in patients with chronic liver diseases. Mechanically, selenium plays a vital role in maintaining healthy liver function and synthesis of essential liver enzymes [21, 22]. Selenium protects the liver mainly in the following aspects: (1) reduces the damage of toxic substances in the environment to the liver;

(2) protects the integrity of the liver cell membrane by scavenging free radicals and preventing lipid peroxides through glutathione peroxidase; (3) accelerates the catabolism of ethanol, protecting the liver from alcoholic damage; and (4) stimulates both the humoral and cellular immunity and enhances immune function against hepatitis virus. The present article reviews the roles of selenium in HCC.

2. The preventive role of selenium in HCC tumorigenesis

One of the most recognized roles of selenium is to prevent cancer. Since the 1970s, the tumorigenesis prevention role of selenium has also been studied in various chemical carcinogen-induced HCC models; for example, selenium can significantly suppress liver cancer carcinogenesis induced by aflatoxin B1, dimethyl azobenzene, or acetylamino. Consumption of food with Aflatoxin B1 (AFB1) contamination is one of the leading causes of liver cancer. In 1985, Milks, et al. investigated the effect of selenium on aflatoxin hepatocarcinogenesis in the rat model and found that oral supplementation of selenium can dose- and time-dependently reduces aflatoxin B1-induced γ-GT variant hepatocyte foci and tumor nodules in rat liver [23]. Similarly, another animal study by Lei et al. in 1990 also found that selenium supplementation had an inhibitory effect on the initiation and promotion stages of AFB1-induced preneoplastic foci and nodules in HCC model without evidence of toxicity [24]. Further study showed that oral supplementation of selenium could dose- and time-dependently reduces aflatoxin B1-induced γ-GT variant hepatocyte foci and tumor nodules in animal models. Shi et al. demonstrated that selenium could reduce the amount of AFB1-DNA binding and effectively inhibit AFB1-induced DNA damage [25]. The main reason may be that AFB1 binds to glutathione (GSH) under the catalysis of the glutathione-S-converting enzyme (GSTS) and is excreted in a non-toxic form, thereby reducing the formation of AFB1-DNA compounds.

Diethylnitrosamine (DENA)-induced and phenobarbital (PB)-promoted HCC model is one of the most popular chemically induced HCC models used [26]. Thirunavukkarasu et al. conducted various studies investigating the chemopreventive properties and biochemical mechanisms of sodium selenite supplementation in the DENA-initiated and PB-promoted HCC rodent model with four parts per million (p.p.m.) of sodium selenite in drinking water for 14 weeks. Selenium supplementation elevates malate dehydrogenase level, a vital enzyme of the citric acid cycle [27]; increases superoxide dismutase (SOD and catalase (CAT) levels, two key antioxidant enzymes [28, 29]; decreases glutathione transferase (GST) level [30]; decreases alanine transaminase (AST), aspartate transaminase (ALT), and lactate dehydrogenase (LDH) elevations [31]; suppresses the elevated glycoprotein levels of glycoproteins such as globulin and hexosamine [32]; elevates ATPase enzymatic level and alters serum mineral levels [33, 34]; as well as increases vitamins C and E [35] in the DENA-initiated and PB-promoted HCC rodent model.

A more recent study by Liu et al. investigated the effects of selenium-enriched malt (SEM) on hepatocarcinogenesis, paraneoplastic syndrome, the hormones regulating blood glucose, vascular endothelial growth factor (VEGF), and several relevant angiogenic cytokines in DENA-induced HCC rat model [36–38]. The results showed that SEM decreased several liver enzyme levels, including alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as gamma-glutamyltranspeptidase (GGT); increased glucose levels and reduced hypoglycemia; and inhibited the angiogenesis by downregulating the expression of VEGF and interacted with tumor necrosis factor-alpha (TNF-α) and insulin-like growth factor II (IGF-II), and nitric oxide (NO). These results suggested that SEM may delay the

development of hepatocarcinoma in DENA-induced HCC rat model and partially by the inhibition of angiogenesis [38].

Other preclinical animal models had also been used to study the effect of selenium in liver cancer. Nine or thirteen weeks of sodium selenite (6 p.p.m) supplementation in drinking water prevented the azo dye (3′-MeDAB) hepatocarcinogenesis in male Sprague-Dawley rats [39, 40]. Supplementation with sodium selenite or selenomethionine reduces the focal volume and lower GST level in 2-acetylaminofluorene (2-AAF)-induced hepatocarcinogenesis rat model [41–43]. Sodium selenite suppressed the BOP-induced HCC in Syrian hamsters and induced apoptosis and caspase-3 level [44]. In the transgenic mice model, selenium supplementation also demonstrates anti-hepatocarcinogenesis effect. For example, in CBA mice, prenatal administration of selenium significantly decreased the spontaneous liver tumorigenesis [45]; in TGF-α/c-Myc transgenic mice, dietary selenium supplementation inhibited hepatocarcinogenesis, promoted apoptosis, and suppressed cell proliferation [46]; in Mdr2 knock-out mice, selenomethionine suppressed the development of HCC by regulating various genes involved in inflammation and oxidative stress [47]. In HCC xenograft, selenium-enriched green tea extracts also showed a suppressive effect in human hepatoma HepG2 cell mice xenograft model [48]. These results suggested that selenium holds a significant promising role as a potential anti-cancer agent *in vivo* in various liver cancer models.

3. Anti-cancer effect of selenium and the potential underlying mechanism

Since the 1970s, the potential anti-tumor effects of selenium have attracted considerable research attention. In vitro studies have shown proliferation inhibitory role of selenium in liver cancer, colon cancer, gastric cancer, etc. [49, 50]. Various *in vivo* and *in vitro* studies have revealed its promising anti-tumor role breast cancer, lung cancer, colorectal cancer, etc. [51, 52]. These results suggest the broad-spectrum anti-cancer effect of selenium. In recent decades, increasing studies have investigated the potential role of selenium in liver cancer, involving several major cancer-associated signaling pathways, metabolic pathways, and transcription factors.

4. Anti-proliferative and apoptosis induction role of selenium in HCC

One of the earlier studies by Baker et al. in the 1990s on human hepatoma Hep3B cell line showed that selenium supplementation restores glutathione peroxidase mRNA expression in selenium-deprived cell culture [53]. Hill et al. investigated in human liver cancer HepG2 cell line showed that in selenium-deprived HepG2 cells, selenoprotein P release decreased to 10% [54]. Further, various studies consistently reported apoptosis induction effect of selenite in human hepatoma cells HepG2 cells, potentially by inducing the release of lactate dehydrogenase (LDH) and decreasing glutathione (GSH) production [55–57]. Another study reported that selenite-induced apoptosis in HepG2 cells was mediated by reactive oxygen species (ROS) that activated JNK to regulate apoptosis [58]. A more recent study on selenium nanoparticle surface decorated with galangin can induce apoptosis through p38 and AKT signaling pathway in HepG2 cells [59]. Similarly, selenium nanoparticles synthesized with extract of hawthorn fruit also induced apoptosis in HepG2 cells [60]. Various mechanisms may be involved in the apoptosis induction

role of selenium and selenium-containing inhibitors for HCC. Tagaram et al. reported a selenium-containing MAPK and PI3 kinase inhibitor, the *Se*, *Se′*-1,4-phenylenebis(1,2-ethanediyl) bisisoselenourea (PBISe), possesses anti-proliferative and pro-apoptotic ability in HCC cell lines *in vitro* and in *in vivo* in a spontaneous murine HCC model [39]. PBISe promoted apoptosis by inhibiting PI3K, MAPK, and STAT3 signaling *in vitro* and with significant reduce tumor sizes *in vivo* ($p < 0.007$) with a significant reduction in tumor survival marker PCNA and angiogenesis markers Vegf-A, Vegf-R3, and CD34 [39]. These results demonstrate the chemotherapeutic effects of selenium by inhibiting tumor proliferation and inducing tumor apoptosis for HCC treatment.

5. Anti-oxidation effect of selenium in HCC

Oxidative stress is characterized by the excessive production of oxidants or ROS and insufficient elimination by protective antioxidants [40]. This persistent imbalance may lead to somatic mutations and neoplastic transformation [40]. In cancer, ROS is commonly found elevated and is a key constituent to cancer survival [61, 62]. Most of the effects of dietary selenium on oxidation are attributable to the insertion of this element to selenoproteins, mostly its cofactor glutathione peroxidase. In the active oxidative metabolism, selenium-dependent glutathione peroxidase acts with tripeptide glutathione (GSH) and competes for the catalyzation for hydrogen peroxide as a substrate, scavenging reactive oxygen species (ROS) and lipid peroxide (LPO) [63]. In 2007, a study by Katzenellenbogen et al. investigated the effect of selenomethionine on oxidative stress and inflammation or lipid metabolism with cDNA microarrays in HCC development in Mdr2 knockout mice model [47]. The results showed that selenomethionine alters the expression of commonly upregulated genes found in response to inflammation, oxidative stress, and cancer [47]. Further, the inhibitory effect on gene expression of selenomethionine is positively correlated with its role to reduce the incidence of large tumors [47]. These results provide a theoretical basis for the anticancer mechanism of selenium.

6. Anti-metastasis effect of selenium in HCC

HCC is characterized by its invasive and metastatic potential [64]. In liver cancer, it has been suggested that SBP1, a Se-containing protein, and its primary function is Se transport, plays a role in metastasis and if found to be highly expressed in healthy liver tissue but was nearly non-detectable in highly metastatic liver cancer cell lines [65]. Epithelial-mesenchymal transition (EMT) is a process that plays a vital role in HCC metastasis cascade and had been suggested to be closely related to the initiation of HIF-1α [64]. SBP-1 is a downstream target of HIF-1α and has been found that loss of SBP1 promoted liver cancer cell migration and increased GPX1 activity, which further suppresses in hydrogen peroxide and other reactive oxygen species, leading to the inhibition of HIF-1α [64]. Recently study by Gao et al. identified 186 differentially expressed genes among control and SBP1 expressing HCC cells [66]. Further investigation showed C-X-C motif chemokine receptor 4 (CXCR4) expression was inhibited by SBP1 and is closely related to the migration and invasion ability of HCC cells through activation of AKT signaling [66]. These results suggested the potential application of selenium in liver cancer metastasis prevention and treatment; however, more data from *in vitro* and *in vivo* are warranted to solidify effect and mechanism of action.

7. Epidemiological investigations and clinical trials

Many studies have shown that the level of blood selenium in patients with chronic liver disease is reduced, and it can be corrected by selenium supplementation [67–69]. Selenium supplementation can inhibit the lipid peroxidation of immune cells in patients with liver diseases, regulate cellular immune function, and alleviate immunopathological damage. In the treatment of patients with chronic hepatitis, in the anti-viral, immune-modulating, and liver-protecting treatment, it is beneficial to correct the blood selenium level of hepatitis patients by detecting and conditioning so that lipid oxidation and liver repair could be resisted [12]. Selenium and selenium supplementation for the treatment of liver disease should attract the attention of the medical community. However, controversies remain with whether a relationship exists between serum selenium level and HCC risk.

Some pioneering studies performed in China in the 1990s showed that plasma selenium level is negatively correlated with the occurrence of HCC in areas with low environmental selenium level [19, 20]. A preliminary report by Yu et al. in 1991 of two intervention trials in high-risk populations of PLC with selenium supplementation in Qidong, Jiangsu province, China, showed that among individuals with a family history of PLC, oral supplementation of 200 mg selenium in the form of selenized yeast (Se-yeast) daily for 4 and 2 years, respectively, showed significant reduction in PLC incidence compared with placebo [19]. Another study by Yu et al. in 1997 of an interventional trial among 130,471 individuals living in Qidong Country showed that a reduction PLC incidence by 35.1% in selenite table salt supplemented group vs. the non-supplemented group after an 8-year follow-up. Consistently, an epidemiological study in Taiwan by Yu et al. involving a cohort of 7342 chronic carriers of hepatitis B and C virus male with an average of 5.3 years of follow-up showed an inverse association between plasma selenium levels and the risk of HCC among men with chronic hepatitis virus infection [70]. Another study in Korea by Kim et al. in 2012 also reported an apparent correlation between low plasma selenium level and incidence of HCC in Korean hepatoma patients [71]. However, no relationship was observed between plasma selenium concentration and incidence of HCC in a study with Japanese hepatoma patients [72]. In 2016, a systemic review meta-analysis involving 9 trials and a total of 1433 subjects supported an inverse correlation between Se level and the risk of HCC, and lowered Se level had a relationship with HCC with a remarkable heterogeneity ($I2 = 74.3\%$, $P < 0.001$) [4]. However, epidemiological investigations and biological studies should be further conducted to demonstrate and verify whether selenium supplements are beneficial for the prevention and treatment of HCC and to elucidate its exact mechanism of action.

8. Discussion and conclusion

Over decades, an increase in the trend of development of novel selenium nanoparticles and selenium-containing inhibitors aims to improve the therapeutic efficacy and relative potency of selenium. Experimental studies with animal tumor models and epidemiological studies of human tumors have revealed that selenium is one of the factors affecting the risk of cancer. The huge number of publications has suggested the potential role of selenium and redox-active selenium compounds as inhibitors and therapeutic agents for liver cancer. However, the studies are difficult to compare among different selenium compounds due to a high degree of variations on the effective dosage anti-proliferation due to the difference in the *ex vivo* culture condition.

Further, although some epidemiology studies have supported the hypothesis that selenium supplementation can reduce the risk of cancer, inconsistent results have also been reported. Another important aspect of being considered for the use of selenium-containing compounds in anticancer is as a chemical protective agent against toxic side effects of anticancer drugs. Selenium has also been reported to have a protective effect against cisplatin-induced nephrotoxicity without affecting its anti-tumor activity in rodent model [73]. Further trials are needed to confirm the selenium supplementation for liver cancer in terms of its effect on prognosis as well as potential toxic effect.

This chapter summarizes the pharmacological effects of selenium in HCC *in vitro* and *in vivo*, and to highlight the recently discovered molecular mechanisms, to outline the correlation of selenium level and the risk of HCC in patients, and to discuss the clinical application of selenium in HCC prevention and treatment. In conclusion, the preclinical studies presented in this chapter summarized the promise of selenium as a potential anti-cancer agent and presented the chemo-preventive role in HCC. Although substantial evidence for the anti-cancer effect of selenium is discussed, further studies are warranted.

Author details

Chien-Shan Cheng, Ning Wang and Yibin Feng*
School of Chinese Medicine, The University of Hong Kong, Hong Kong

*Address all correspondence to: yfeng@hku.hk

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