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Current therapeutic modalities and chemopreventive role of natural products in liver cancer: Progress and promise

Chemopreventive role of bioactive natural products in Liver cancer

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Abstract

Liver cancer is a severe concern for public health since the clinical cases are increase each year, with an estimated 5-year survival rate of 30-35% after diagnosis. Hepatocellular carcinoma (HCC) constitutes a significant liver subtype of cancer (approximately 75%) and is considered primary liver cancer. Treatment for liver cancer is mainly dependent on the stage of its progression, where surgery including, hepatectomy and liver transplantation, and ablation and radiation therapies are the prime choice. For advanced liver cancer, various drugs and immunotherapy are also used as first-line treatment, whereas second-line treatment includes chemotherapeutic drugs from natural and synthetic origins. Sorafenib and Lenvatinib are first-line therapies, while Regorafenib and Ramucirumab are second-line therapy. In recent years, various metabolic and signalling pathways such as Notch, JAK-STAT, hippo, TGF-β, and Wnt signaling pathways have played a critical role during HCC progression; hence studied in detail found to be targeted by these treatments are required. Dysbiosis has also been implicated in liver cancer. Drug-induced toxicity is a key obstacle in the treatment of liver cancer, necessitating the development of effective and safe medications, with natural compounds such as resveratrol, curcumin, diallyl sulphide, and others emerging as promising anticancer agents. This review highlights the current status of liver cancer research, signalling pathways, therapeutic targets, current treatment strategies and the chemopreventive role of various natural products in managing liver cancer.

Key Words: Liver cancer; hepatocellular carcinoma; signalling pathways; therapeutic targets; natural products; Chemopreventive.

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Core Tip: Liver cancer is a serious public health concern and its therapy is stage-dependent. Approximately 75% of all liver cancers are hepatocellular carcinomas, which are regarded as primary liver cancers. First and second-line therapies are used to manage the disease but they have their own limitations in terms of toxicity and other severe side effects. Natural products are the prime choice for future treatment of liver cancer. With the advancement in the knowledge about molecular mechanism of the disease, newer strategies having lesser side effects and greater effectiveness are the need of time.

INTRODUCTION

Liver, the human body's largest solid organ, has pivotal roles in removing various blood toxins and maintaining bioenergetics and cellular metabolism [1, 2]. The liver is structured into four lobes which are made up of multiple lobules, each having a flowing duct toward the common hepatic duct, responsible for bile excretion [3]. Changes in lifestyle patterns and excessive use of medicines, alcohol and intake of various non-hygienic supplements, further imposed stress and finally damage the liver [4, 5]. Over-dosed alcohol and viral infections causing hepatitis are the critical factors for liver cancer clinical cases [6, 7]. Each year approximately 0.8 million new clinical cases of liver cancer are diagnosed. Additionally, from this disease about 8,30,180 people died worldwide in 2020 alone, and this figure seems to be increasing day by day, as per world health organization (WHO) surveillance reports [8]. Among various liver cancer types, hepatocellular carcinoma (HCC) is the most common type and accounts for approximately 85% of primary liver cancer cases and often occurring in people having chronic liver diseases. It is the most common and second leading cause of cancer-related deaths in Asian and sub-Saharan African countries. It is sixth most common in western countries due to escalating hepatitis C burden along with non-alcoholic steatohepatitis (NASH) and obesity [9, 10]. Additionally, in patients with a preclinical history of chronic liver diseases and cirrhosis, the development of HCC is a complex process, including

inflammatory damage leading to hepatocyte necrosis, regeneration and fibrotic deposition [11, 12].

In recent years, multiple efforts have been made to manage HCC using various chemotherapeutic approaches, where targeted tyrosine kinase inhibitors, immunotherapy and anticancer combination therapies are the main ones [13]. However, chemoresistance, initiation and progression of tumors mainly reprogram the cellular metabolism, particularly during HCC development [14]. These metabolic alterations are key factors promoting tumor growth, proliferation and requirements of cancer cells, such as increased energy production, macromolecular biosynthesis and maintenance of redox balance. Since, liver is main site for the contact with a variety of orally ingested therapeutic drugs, alcohol and other xenobiotics after intestinal absorption where this organ is susceptible to various chemicals [15, 16]. These chemicals augment serious complications such as acute and chronic hepatitis, granulomatous hepatitis, cholestasis with or without hepatitis, tumours and vascular disorders [17].

Among various factors responsible for HCC, hepatitis viral infection and resulting cirrhosis cover a significant portion of clinical numbers. Various viral infections cause up-regulation of hexosamine and membrane lipid biosynthesis, by modulating glutamine-fructose-6-phosphate transaminase 1 (GFAT1) and choline kinase A (CHKA) expression [18, 19]. These findings have been further validated with some scientific results where GFAT1 is up-regulated in HCC patients and its over-expression enhances tumorigenic phenotypes as observed during *in vitro* studies [20]. HBV also alters lipid metabolism where viral proteins are known for inducing lipid accumulation *via* the upregulation of sterol regulatory element-binding protein 1 (SREBP1), peroxisome proliferator-activated receptor γ (PPAR γ) as well as lipogenic and adipogenic enzymes, which are also reported during HCC progression [21, 22]. In addition, HCC cells infected with HCV are also known to exhibit altered glycolysis and gluconeogenesis along with the activation of lipid-metabolism transcription factor PPAR γ in human hepatocytes, similar to HCV infection [23]. Some early findings of HCC also reported the CD36 gene

role in the free fatty acid uptake and its increased expression during chronic alcohol consumption, thus modulating lipid metabolism, up-regulation of SREBP1c and PPAR γ, and down regulation of SIRT1; this collectively lead to impaired fatty-acid oxidation [24, 25]. Interestingly, non-alcoholic fatty liver disease (NAFLD) also manifests alterations in mitochondrial and other metabolic pathways that are reminiscent of HCC metabolism. As mentioned earlier, modifications in the processes are primarily analogous to many contexts observed in HCC. However, still there is a need for a better understanding of various underlying mechanisms governing metabolic changes during HCC.

Surgical resection is the primary choice for treating HCC, where the recurrence rate and metastasis mostly occur, thus limiting a proper treatment for HCC. Due to the minimal number of drugs available for the treatment of HCC, chemotherapy still remained insufficient for the successful management of HCC [26, 27]. Although the first-line and second-line therapies can increase the life span for some months, these have serious side effects and resistance problems [28]. Since natural products are promising and cost-effective against various health ailments, it seems reasonable to focus on HCC management using natural products where the anticancer drugs for HCC are limited [2, 4, 29]. This review focuses HCC and its associated pathways, descriptive illustration of various natural products, along with their anticancer properties. This review provides an informative platform with updated literature to look further regarding liver cancer, signalling pathways, therapeutic targets, current treatment strategies and the chemopreventive role of various natural products.

LIVER CANCER

Molecular signalling pathways associated with hepatic cancer

The liver is highly exposed to the foreign material, and their continuous processing is required for body's normal functioning. In addition, alcohol consumption imposes stress on hepatic cells. This condition worsens when combined with a genetic defect in hepatic cells. These factors, either alone or in combination, alter the molecular signalling

events responsible for controlled cellular proliferation and differentiation, ultimately leading to hepatic cancer [23]. Targeting these signalling pathways by therapeutic molecules is an important strategy. Inhibition of hepatic cancer-associated signalling pathways ameliorates cancer hallmarks such as increased cellular proliferation, reduced apoptosis, migration, angiogenesis, etc [24, 25]. This section discusses recent advances in molecular signalling pathways associated with the different stages (initiation and development) of liver cancer and their therapeutic target potential. Critical signalling pathways related to the hepatocellular carcinoma include transforming growth factor-β (TGF-β), Wnt/B-catenin, Hedgehog (Hh), Notch, Epidermal growth factor (EGF), Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Janus kinases (Jak)/Stat3, and Hippo signaling pathways [30]

The human liver possesses regeneration potential, and highly controlled molecular mechanisms regulate its repair and regeneration. The notch signaling pathway is involved in the repair and regeneration of the liver, but its malfunction (loss or gain of function) is highly associated with hepatic diseases, including cancer [31]. Notch1 up expression has been found in most hepatic cancer patients. Molecular profiling studies revealed notch target genes such as Hes1 and Hey1 in hepatic cancer patients with increased cellular proliferation, reduced apoptosis, increased metastasis and angiogenesis in hepatic cancer cells [32, 33]. Besides this, notch signaling cross talk with other molecular pathways (such as hypoxia signalling), which are known to be associated with hepatic cancer [34]. Cytokine signalling pathway such as JAK-STAT (Janus kinase-signal transducer and activator of transcription) have been involved in viral escape in virus-induced hepatocellular carcinoma [35]. Viral invasion and liver injury stimulate hepatocytes and kupffer cells to secrete SHH ligands. The ligand triggers Smoothened (Smo) receptor by interacting with the Patched protein which ultimately initiates the hippo signaling pathway in hepatic cancer cells. The activation of the hippo signaling pathway results in the increased transcription of the effector gene (cyclin D, c-Myc, MMPs, and CD133 *etc.*), affecting cell proliferation, invasion, and stemness properties of hepatic cancer cells [36-38].

TGF-β signaling pathway promotes epithelial to mesenchymal transition, angiogenesis, macrophage maturation, cancer stem cell population, and cellular proliferation in hepatocellular carcinoma. Cross talk of TGF-β with other pathways (EGF, Wnt, SHH *etc.*) is associated with liver cancer [39, 40]. Increased Wnt ligand expression and/or mutation(s) in molecular components of Wnt signalling pathway results in hyperactivation of the pathway in hepatic cancer cells. The binding of the Wnt ligand to its receptor followed by the production of free β-catenin and its translocation to nucleus, ultimately activates the transcription of target genes (CD44, EpCAM, cyclin D1, c-Myc, *etc.*) [41]. Transcription of target genes ultimately increases cellular proliferation, stemness, angiogenesis and migration potential in hepatic cancer cells. Wnt signaling response to a hypoxic condition in the tumor microenvironment increases stemness potential in hepatic tumor cells. Like other solid tumors, liver cancer cells secrete various growth factors such as platelet-derived growth factor-PDGF, fibroblast growth factor-FGF, hepatocyte growth factor-HPF, and vascular endothelial growth factor-VEGF. These factors in turn induce angiogenesis to ensure the appropriate supply of nutrient and oxygen. Mostly, hepatic cancer is result of chronic liver cirrhosis which ultimately takes the shape of advanced hepatic cellular carcinoma. Available clinical data showed that the percentage protein mutation increased as the disease travelled from the initiation stage to highly advanced cancer stage [42]. Mutation in TERT gene (catalytic subunit telomerase reverse transcriptase) has been associated with the increased cellular proliferation in liver cancer cells. Clinical data revealed that TERT promoter mutation increased up to ten times in hepatocellular carcinoma cells compared to low grade dysplastic nodules [43]. It indicates that mutation plays an important role in the initiation and progression of the pathological stage of hepatocellular carcinoma. Besides, other mutations are only involved at the later stage of the disease progression and produce more genetic diversified subtypes [44].

Recent development in therapeutic targets in hepatic cancer

Sorafenib (SB) is a first line chemotherapeutic agent approved for advanced hepatocellular carcinoma. It is a multikinase inhibitor targeting Raf, EGFR, VEGFR, PDGFR, FLT3 (FMS-like tyrosine kinase-3) and c-kit [45, 46]. Clinical studies revealed that SB treatment inhibited hepatic tumor growth and angiogenesis in advanced stage, but it's prolong exposure induces resistance in patients [47-50]. Recently it has been reported that the second line drugs such as lenvatinib, regorafenib, and ipilimumab produced better therapeutic outcome, and increased over all disease free survival in liver cancer patients [51]. Increased tumor growth and distance metastasis in SB resistance patients and lesser overall survival rates in SB treated liver cancer patients necessitate exploring newer and potential therapeutic targets in liver cancer. Besides, exploration of newer therapeutic agents and combinatorial drug regimens may also be explored to target the disease and increase the therapeutic outcome in patients. The current treatment strategy for liver cancer (first and second line of therapies) is discussed in more detail in the subsequent section of this review.

Recently *Luo et al* identified emerging targets in liver cancer by utilizing comprehensive and integrated multi-omics analysis. The study identifies potential signaling pathways (Tp53/RB1, Wnt/β-catenin, PI3/Akt/mTOR, JAK/STAT, MAPK and TGF-β) and molecular events (telomere maintenance, cellular differentiation, chromatin remodeling and oxidative stress) in liver cancer. Mutation mediated protein (CCND1, CTNNB1, TERT, PIK3CA, KRAS, KEAP1, NFE2L2, JAK3, FGF4, FGF19, and FGF3) and inactivation (TP53, Rb1, CDKN24, CHN2B, ATM, AXIN1, APC, ZNRF3, HNF1A, APOB, ALB, ARID1A/B, ARID2, SMARC2, BAP1, BRD7, KMT2C, PTEN, TSC1, TSC2, RPS6KA3, and ACVR2A) activation are associated with the pathophysiology of liver cancer and emerged as therapeutic targets for hepatic cancer [52]. β2-spectrin (SPTBN1), a cytoskeleton protein is essential for the developing various organs, including the liver. It performs both structural (establishment and maintenance of cellular structure) and functional (apoptosis, cell adhesion, and cell cycle regulation) role [53]. Recently it has been reported that SPTBN1 induces lipogenesis mediated liver cancer in high-fat diet fed experimental mice. The study proposed SPTBN1 as a potential therapeutic target for

liver cancer [54]. Recently, *Craig et al* studied the expression profile of cancer/testis antigens or CTA proteins in hepatocellular carcinoma. The study showed that CTA was overexpressed in HCC patients and associated with poor overall survival and prognosis. Further experimental evidence of the study showed that MAGE-A (melonoma-associated antigens family A), a member of CTA family protein is responsible for increasing cellular proliferation, decreased apoptosis, and aggressiveness in hepatocellular carcinoma experimental models. The study revealed that MAGEA3 is involved in the developing hepatic carcinoma and could serve as a potential novel target for the disease [55]. Glypican-3 (GPC-3), a heparin sulfate proteoglycan, aresignificantly overexpressed in >80% of HCC patients and was positively associated with the poor diagnosis in the patients [56, 57]. Clinical studies showed that targeting GPC-3 by developed antibodies significantly increased disease progression-free survival in patients having over-expressed GPC-3 in comparison to patients with low GPC-3 Levels. Combination of chemotherapy and the immunotoxin (antibody + exotoxin) mediated GPC-3 targeting showed better therapeutic outcomes in liver cancer patients [58-60]. These facts indicate the promising therapeutic potential in GPC-3 proteins in liver cancer. Interaction between hepatocyte growth factor (HGF) and its receptor c-Met is an important incidence in liver regeneration. Over expression and/or mutation, in c-kit have been positively associated with the liver cancer [61]. Direct or indirect (via different signalling pathways) interaction among HGF and c-kit increases the cellular growth, angiogenesis and metastasis in liver cancer cells [62]. Pre-clinical and clinical study reports that interrupting the association between HGF and c-kit results in potential therapeutic response in liver cancer [63-65]. Thus HGF and/or c-kit are potential therapeutic targets in liver cancer. Various studies showed that cancer cells rewire their metabolic pathways to fulfil their increased need for nutritional requirement. Liver cancer cells also reprogram their lipid metabolic pathway to combat their increased nutritional requirement, which ultimately help in cellular proliferation, growth, and survival. Pre-clinical studies showed that biosynthesis of lipids and desaturation process play an important role in liver cancer initiation, progression and

survival. Recently, *Pope et al* beautifully reviewed aberrant biochemical/molecular players of lipid metabolism as potential therapeutic targets in liver cancer [66, 67]. Over expression of lipid metabolism enzymes such as fatty acid synthetase, ATP citrate lyase, stearoyl-CoA desaturase-1, and acetyl CoA carboxylase have been associated with the various cancers including liver cancer. Targeting these enzymes with small molecules showed a potential tumor-suppressive nature in experimental models of liver cancer. There is a need to study some enzyme inhibitors in clinical trial, such as SCD-1 (Stearoyl-CoA desaturase-1) inhibitors [68-70].

MicroRNAs (miRNAs) are short length non-coding RNAs involved in regulating gene expression and thus control the normal physiology and disease pathophysiology by normal and abrupt expression, respectively [71]. Modulating miRNAs by therapeutic molecules, and/or using their respective inhibitors or mimics is an important strategy to target cancer at gene level [72]. The study showed that aberrant expression of miRNAs (miR34, miR36, miR21, miR203, miR17, miR83, miR 93, miR221, *etc.*) in liver cancer cells is associated with the increased cellular proliferation, metastasis, angiogenesis, drug resistance, cell survival and reduced apoptosis [72]. MicroRNA-based mouse model of hepatocellular carcinoma has been developed to study the inflammation, tumor initiation, metabolic alteration, and hepatocyte differentiation [73]. Recent studies showed the therapeutic potential of miRNAs in liver cancer by utilizing the miRNA inhibition/replacement approach. A recent study identified miRNAs (miR-550a, miR-574, miR-424, let-7i, miR-549, miR-518, and miR-512) as significantly associated with the overall survival in liver patients using bioinformatics tools which indicates their therapeutic potential. The study proposed that these miRNAs should be studied in detail for their therapeutic potential in liver cancer experimental models [74]. In another recent study, *Dai et al* compared the publically available liver cancer miRNA expression data with the human hepatocellular carcinoma (with Hepatitis B positive and negative) data (generated by the study group). The study identified miR-0308-3p as novel miRNA associated with the HBV-positive hepatocellular carcinoma. Further the mechanistic experiment showed that the miRNA suppresses hepatic cancer cell proliferation and

arrests cells in G1/S phase by targeting CDK6 and cyclin1 genes [75]. These results showed that the miR-0308-3p is a novel therapeutic target in liver cancer. Interestingly, *Shao et al* developed personalized miRNA cocktail therapy by combining nanotechnology and gene therapy to treat liver cancer. The research group encapsulated mimics (of miR-199a/b-3p) and inhibitor (of miR-10b) into a polymer-based nanoplatform (PCAPC). The *in vitro* and *in vivo* experiments showed the better anticancer potential of the PCACP/miR-cocktail system in comparison to mimic or inhibitor treatment alone in liver cancer experimental models [76]. This study showed a novel potential strategy to treat liver cancer by combining nanotechnology and gene therapy. In a different study, *Wang et al* studied the relation between LINC01018 (a long non-coding RNA), miR-182-5p and FOXO1 protein in the hepatocellular carcinoma patients sample. Result showed poor expression of the long coding RNA and FOXO1, and higher expression of miR-182-5p in the carcinoma patient samples. Further the forced expression of LINC01018 under *in vitro* and *in vivo* experimental model showed decreased cellular proliferation and induced apoptosis with the increased miR-182-5p levels. The study showed liver cancer therapeutic potential in LINC01018 by miR-182-5p sponge mediated down regulation of FOXO1 expression [77].

Current treatment strategies for liver cancer

The dysregulated cell cycle, apoptosis, and many other key signalling pathways are linked to HCC pathogenesis. Chemotherapeutic approaches similar to different types of cancer are also reported with a limited number of drugs for the cure of HCC and various side effects. Sorafenib, an oral multi-targeted tyrosine kinase inhibitor has been used as first-line treatment for advanced HCC showing increased survival of approximately 12 mo compared to controls [78]. Various antiangiogenic agents such as bevacizumab (human monoclonal antibody directed against vascular endothelial growth factor) and erlotinib (an epidermal growth factor receptor tyrosine kinase inhibitor) have also been studied and shown effective results in early studies [79]. Until 2016, sorafenib was the only FDA (food and drug administration, USA) approved first-

line treatment for HCC whereas lenvatinib has also been identified and is in use for advanced HCC [80]. Sorafenib acts as an inhibitor of intracellular tyrosine and serine/threonine protein kinases such as vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), c-Rafand b-Raf MAP kinases, this in turn induces autophagy. Due to drug resistance and side effects such as liver fibrosis, clinical usage of sorafenib is also limited [63]. Long-term exposure to sorafenib also induces cancer cells with less E-cadherin content making them more invasive. Some second-line treatments are also available for HCC, including regorafenib, ramucirumab and cabozantinib, which are rarely used and are less efficient [28, 51, 79]. It is presented that chemotherapeutic drugs used for HCC treatments are limited in number and seems to be less effective, considering their efficacy, bioavailability and side effects. Considering the side effects of ongoing therapies, scientific pieces of evidence are also suggestive for the use of natural products for the management of HCC, since they can inhibit viral infection, inflammation, oxidative stress, metabolic disorders, angiogenesis and metastatic activity, which are known as prime contributors for HCC [2, 80, 81]. Hence there is strong demand for searching novel plant-based drugs for managing HCC with lesser side effects and chemo-toxicity. Therefore, several drugs are used to treat HCC to target the inhibition of some of these processes (Table 1).The current therapeutic interventions for patients with HCC are divided into first-line and second-line therapies. The pharmacological features of these drugs are discussed in the following section of this review.

First line therapies

Sorafenib

Sorafenib (BAY 43-9006, Nexavar) is the first-ever systemic drug as well as a standard therapeutic agent approved by the United States Food and Drug Administration (US FDA), for the treatment of liver cancer patients who just don't fit for surgical resection or liver transplant [79]. Sorafenib was the only first-line treatment in last ten years until

the US FDA approved lenvatinib as a frontline therapy in 2018. It is a tyrosine kinase inhibitor that targets vascular endothelial growth factor receptor family (VEGFR 1 and VEGFR 2), platelet-derived growth factor receptor (PDGFR-β). It activates adenosine monophosphate activated protein kinase (AMPK) that can block the formation of tumor blood vessels and inhibit the proliferation of liver cancer cells [82]. For individuals with HCC, sorafenib has a clear advantage in terms of survival. Sorafenib improved overall survival considerably compared to placebo in two phase III clinical randomized controlled trial (10.7 mo *vs* 7.9 mo and 6.5 mo *vs* 4.2 mo). However, the side effects associated with these clinical trials were diarrhoea, tiredness, and hand–foot skin response [80, 82].

However, several variables hinder more people from getting benefited post sorafenib treatment. Because of the genetic variability of HCC and other factors, around 40% of people with HCC can benefit from sorafenib. Studies have shown that the drug sorafenib found was more beneficial for some patient categories in several trials. The two clinical studies mentioned above featured only a small number of patients, all of whom had a good liver function. These individuals were termed SHARP-eligible patients, and only SHARP-eligible individuals benefited from sorafenib treatment [79, 82]. Furthermore, the effectiveness of sorafenib is greater in hepatitis C-infected individuals than in others who have always been resistant to sorafenib. Primary resistance is another term for the unclear mechanism of this phenomenon [82, 83]. However, some research has uncovered probable explanations. Gene polymorphism may be a crucial factor influencing sorafenib's function. Polymorphisms in the ATP binding cassette (ABC) subfamily B member 1 (ABCB1), ATP binding cassette subfamily G member 2 (ABCG2), solute carrier family 15 member 2 (SLC15A2) and endothelial nitric oxide synthase (eNOS) have been linked to the action of sorafenib, according to researchers [83]. This was further confirmed by Silvia and co-workers reported findings that β-caryophyllene oxide inhibits ABC proteins and causes HCC cells to become chemosensitizer to sorafenib [84].

Lenvatinib

Lenvatinib (E7080, Lenvima) is an antitumor drug that belongs to quinoline carboxiamides family. The IUPAC name of lenvatinib is 4-[3-chloro-4-(cyclopropylcarbamoylamino) phenoxy]-7-methoxyquinoline-6-carboxamide. Levatinib acts as multikinase inhibitor *via* targeting VEGFR 1-4, PDGFR-α, PDGFR-β, fibroblast growth factor receptor family (FGFR 1-4), tyrosine kinase receptor (KIT) and rearranged during transfection receptor (RET) that subsequently leads to angiogenesis inhibition, and reducing vascular permeability of tumour microenvironment [28]. Lenvatinib is an effective drug that increases the overall survivability in patients suffering with advance HCC and whose tumour cannot be removed through surgery. In phase I clinical trial, Lenvatinib (12 mg and 8 mg) was effective in patients suffering from advanced HCC and Child-Pugh A or B score. However, the adverse effects observed during 12 mg daily lenvatinib oral treatment were hypertension, decreased body weight, loss of appetite fatigue, diarrhoea etc [28, 70]. A Phase II clinical trial was conducted to evaluate the effectiveness of lenivatinib in advanced HCC individuals; with unresectable HCC. The trial was conducted on 46 people who have received 12 mg lenivatinib orally once daily for 28 days, and lenivatinib demonstrated high efficacy with a good toxicity profile. However, the efficacy of the drug lenivatinib was influenced by the patients' body weight [85, 86].

Second line therapies

Regorafenib

Regorafenib (BAY-73-4506) with the brand name Stivagra is an oral multikinase receptor antagonist developed by Bayer and approved by US-FDA in June 2017 to treat unresectable advance liver cancers. Despite its structural similarity with sorafenib, regorafenib showed more effectiveness in inhibiting the activities of various protein kinases associated with neovascularisation (VEGFR 1-3, TIE2), oncogenesis (KIT, RET, Raf1, BRAF) and tumour microenvironment (PDGFR-β, PDGFR-α and FGFR) with better drug tolerance profile [87, 88]. Regorafenib (160 mg/day for 28 days) treated HCC

patients showed better overall survivability i.e., 10.6 mo compared to 7.8 mo in the placebo group in a randomised, double blind, placebo controlled phase III trial. However, the side effect observed in the patient was hypertension, unlike body weight loss, hepatorenal dysfunction, and fatigue as in sorafenib-treated individuals [87, 88].

Ramucirumab

Ramucirumab, sold under brand name Cyramza and others, is a recombinant monoclonal antibody (IgG) that targets VEGF2 and blocks its binding to VEGFR ligands. The anticancer activity of ramucirumab as second-line therapy was evaluated in Phase II clinical trials in advanced HCC patients having a high level of α-fetoprotein biomarker. These trials found that individuals who received ramucirumab had a better overall survival rate than those who received placebo; the medicine was well tolerated and had an acceptable toxicity profile [89].

Future promising therapeutic drugs

Pirfenidone

Pirifenidone (Esbriet®) is an orally administered antifibrotic, antioxidant, and anti-inflammatory drug that has been studied in clinical and preclinical trials to treat hepatic and idiopathic pulmonary fibrosis [86]. Pirifenidone was effective in causing cell cycle arrest at G0/G1, eventually inhibitingcell proliferation in an *in vitro* model. Similarly, it induces apoptosis in HepG2 cells *via* Wnt/β-catenin signalling pathway. Pirifenidone has also been demonstrated to be a potent antifibrotic agent at a dosage of 300 mg/kg in a carbon tetrachloride-induced HCC mice model. However, the cellular mechanisms behind the responses elicited by pirifenidone remain unknown [70, 86]. Table 1 summarises the pharmacological properties of drugs used in liver cancer [86].

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GUT MICROBIOTA AND LIVER CANCER

Multiple lines of scientific evidence have suggested the significant contribution of gut microbes to critical aspects of human health. Even though the gut microbiota offers substantial benefits to the host, particularly in terms of immunity and metabolic activities, there's still growing evidence of the role of gut microbes in several pathological conditions. They promote disease progression not just locally, as in chronic inflammatory bowel syndrome (IBD), but also in other part of the body; liver, brain, heart, *etc.* [91]. Similarly, there is mounting evidence that the gut microbiota plays a significant role in carcinogenesis *via* its local and long-distance impacts. The liver is intimately connected to the gut through the portal vein. The liver is directly exposed to microbial metabolites and microbe-associated molecular patterns (MAMPs) that can induce inflammatory reaction through pattern-recognition receptors (PRRs), and receive nutrient-rich blood from the gut. The multilayer epithelial barrier is responsible for minimal hepatic exposure to MAMPs. Although, as in chronic liver diseases, altered gut barrier and microbiota composition increases the incidence of inflammation and progression of liver disorder and thus raise the risk of HCC [92].

According to accumulating scientific evidence, intestinal dysbiosis appears to have a significant role in the development of chronic liver disease and HCC. Metagenomic studies have demonstrated significant changes in the gut microbiota composition in a variety of chronic liver diseases as well as in people with cirrhosis [93]. Patients with advanced liver disease and cirrhosis have an increase in potentially harmful bacteria and a decrease in microorganisms with beneficial qualities in their gut microbiomes [94, 95].

Toll like receptor-4 (TLR4) is found in various liver resident cells such as Kupffer cells, hepatic stellate cells (HSCs), endothelial cells, and hepatocytes. A study conducted by Dapito and colleagues in bone marrow chimeric mice concluded that the presence of TLR4 on these liver-resident cells promotes fibrogenesis and hepatocarcinogenesis [96]. Lipopolysaccharides (LPS), a gram-negative bacterial cell wall component, are produced through the leaky gut and mainly target Kupffer cell and HSCs, which

appears to increase the incidence of hepatocarcinogenesis. Activation of TLR-4 in HSCs causing nuclear factor kappa beta (NF-κβ) mediated increased expression of epiregulin, a hepatomitogen and belonging to the epidermal growth factor family, and reported to have strong mitogenic potential on hepatic cells [96, 97]. The finding was further confirmed when hepatocarcinogenesis decreased in epiregulin-deficient rats treated with *N*-nitrosodiethylamine (DEN)-CCl4. Another important method through which the LPS–TLR4 axis promotes HCC development is through NFκβ-mediated hepatocyte apoptosis prevention [96, 97].

BIOACTIVE NATURAL PRODUCTS AGAINST LIVER CANCER AND MOLECULAR MECHANISMS INVOLVED

For centuries, bioactive natural products from plants have been extensively used to treat many human diseases. Recent molecular evidences are explaining their modes of action, metabolic regulations, and identification of their biological targets. These evidences add value to their potential use in the chemoprevention of HCC. The promising candidate bioactive natural products are discussed in this section, where their possible role in liver cancer therapy has been reported.

In vitro studies

In the last two decades, growing evidence suggested affirmative role of resveratrol (polyphenolic natural product) in the chemoprevention of liver cancer. Its application is somewhat limited due to its poor bioavailability. Previously, resveratrol was shown to negatively regulate the cellular proliferation of rat hepatoma and human hepatoblastoma cell line HepG2 at 1-150μM concentration [98]. A decreased proliferation and invasion of HepG2 cells and rat ascites hepatoma cell AH109A were also reported. In subsequent studies, resveratrol was identified to induce apoptosis in *in vitro* studies using HepG2and H4IIE rat hepatoma cells [98]. *Notas et al* showed that even a couple of hours treatment of resveratrol (10^{-6}-1 μM) can interfere with DNA replication and causes cell cycle arrest [99]. A recent study by *Roncoroni et al* using SK-ChA-1 human

cholangiocarcinoma cells in multicellular tumor spheroids model showed the arrest of cell cycle at G1/S phase, at a concentration up to 64 µM resveratrol. Resveratrol was shown to limit cellular proliferation and mobility by activating autophagy through p53 and inhibiting phosphoinositide 3-kinase/Akt in MHCC-97H cells. Autophagy was thus explained to increase chemopreventive property of resveratrol [100]. A study on HepG2 and Hep3B cells identified that resveratrol regulates the PTEN/Akt signaling pathway through down-regulation of MARCH1, which ultimately aggravates apoptosis and inhibits cellular growth [101].

A curcumin analogue, namely CUR3d, inhibited the proliferation of liver cancer cells at 100 µM, which was reported to be due to downregulation of PI3K/Akt and inhibition of the NFκB pathway, which were responsible for cancer cell growth [102]. In another study, supplementation of curcumin (1 g/kg) significantly inhibited the growth and liver metastasis of colorectal cancer cells [103]. A recent study showed that microemulsion formulation could improve 1225 times the water solubility of myricetin and enhance its anti-proliferative activity against human liver cancer cells (HepG2) [104].

Extract of immature plum induced extrinsic apoptosis in HepG2 cells as evidenced by caspase-8, -10, and -3 activation as well as DNA fragmentation [105]. Two natural polyphenolic compounds (epicatechin and gallocatechin gallate) were quantified in the extract and might be responsible for the anti-cancer potential [106]. The garlic extracts are consisting of multiple organosulfur components and flavanols that obstruct different stages of the carcinogenic process. Diallyl sulfide is one of the important component has inhibited diethylnitrosamine (DEN) induced HCC. Another constituent of *allium* extracts, S-allyl cysteine (SAC), has established anti-proliferative and metastasis activity in the management of HCC [107]. 6-shogaol and 6-gingerol are the most common active constituents in ginger that displayed an anticancer activity against hepatoma cell line by triggering reactive oxygen species (ROS)-mediated apoptosis and controls the matrix metalloproteinases (MMP)-9 and TIMP-1 expression [108]. In vitro and *in vivo* activities of many natural products are depicted in Table 2.

In vivo studies

Intraperitoneal resvertrol administration (1 mg/kg body weight) for 7 days in AH-130 hepatoma cells implanted male Wistar rats arrested tumor growth. *Liu et al* showed the immunomodulatory role of resveratrol (500, 1000, 1500 mg/hg body weight for 10 days) in BALB/c mice implanted with H22 hepatoma cells [109]. *Rajasekaran et al* studied the chemopreventive role of resveratrol in a model of N-nitrodiethylamine (DEN)-induced HCC in male Wistar rats. It was showed that it induces apoptosis by PARP cleavage, caspase-3 activation, p53 up-regulation and cytochrome-c release when given an early dose of resveratrol (200 mg/kg body weight) [110]. *Gao et al* tested the chemopreventive property of resveratrol in MHCC97-H inoculated athymic nude mice. The study identified its antitumor activity by down regulating the HGF-c-Met signaling pathway [111]. Recent studies have explored the therapeutic potential of resveratrol when conjugated with nanoparticles. Resveratrol-gold nanoparticle (Res-GNP) has shown improved anti-cancer effects than resveratrol alone in Hepg2 cells and xenografted BALC/c nude mice [112].

Lycium polysaccharide portion (LPP) is the utmost crucial part of *Lycium barbarum* that has abundant of biological activities such as antioxidant, neuroprotective, immuno-protection, anti-tumor, and glucose metabolism regulation. The LPP has inhibited the propagation of hepatocytes and leads to apoptosis of liver hepatocytes thus indicating its anti-cancerous role. A clinical trial showed that consumption of LPP juice leads to an elevation in the interleukin (IL-2), immunoglobulin G, serum antioxidants levels, lymphocyte count and diminutions of lipid peroxides levels in human beings [113]. Berberine mediated an anticancer activity by inhibiting anti-apoptotic protein bcl-2, activating caspase cascade and pro-apoptotic pathway activation of Egr1-NAG-1 (non-steroidal anti-inflammatory drug-activated gene). Berberine greatly facilitates the phosphorylation of AMPK, thus increasing the concentration of p-AMPK/total AMPK. The AMPK-mediated mitochondrial/Caspase pathway by raising the Bax/Bcl-2 ratio may be responsible for the anticancer activity of berberine. A recent study suggested that long-lasting polyethylene glycol-based liposomal berberine displayed *in-*

vivo and *in-vitro* anti-HCC activity [114]. Researchers established paclitaxel-loaded nanoparticles, followed by galactosamine conjugation on the formed nanoparticles, found to be effective in reducing the tumor size through apoptosis activation and cell cycle arrest [115]. The efficacy of many natural products against liver cancer is shown in Table 2.

<Insert Table 2>

CHALLENGES AND WAY FORWARD IN NATURAL PRODUCT BASED ANTI-LIVER CANCER THERAPEUTICS

Natural products have become the point of attraction in anti-cancer drug discovery due to unsolved problems related to the present chemotherapy such as drug resistance and toxicity. It should be noted that from 1940 to 2014, about 50% of the small molecules approved for cancer(s) treatment were either natural compounds or their derivatives [129, 130]. On the other hand, natural products based on anti-cancer therapy have also been suffering from some therapeutic limitations, which majorly concerns therapeutic outcome, lower bioavailability, selected and targeted delivery *etc.* This section highlights these issues, recent advances in the field, and future promises. Advancements in computational biology/pharmacology/chemistry and high-throughput *in vitro* screening of natural anti-cancer drugs highly accelerated the drug discovery process, ultimately resulting in a lead molecule. Most of the time, it is frustrating to get non-satisfactory activity of the lead natural molecule in *in vivo* experiments and/or clinical studies, which results in lesser activity and non-selectivity for a given therapeutic target. It has been proposed that delivering the natural product(s) to a targeted site using an appropriate delivery system may improve the efficacy by increasing their bioavailability. Moreover, the process may also decrease the off-target effects and toxicity related issues in a given therapy [131]. Different means of

drug delivery tool or appropriate vehicle has been exhaustively discussed elsewhere [132]. Use of these tools/vehicles is very much dependent on their biocompatibility, degradability and functional limitations. However, the concept is quite promising but possesses its limitation (fast elimination from the body, toxicity and inflammation), which still need to be addressed by the scientific community [132, 133].

Considering the efficacy of the natural product in living system, it is very important to understand the drug's pharmacokinetics. Any parameter *viz.,* absorption, distribution, metabolism and excretion, may primarily affect the therapeutic outcome of the natural product-based drug. Absorption of a particular drug is highly influenced by the mode of drug administration, i.e., whether it is oral, intravenous or through inhalation mode. In each case, the drug shows a different kinetic behaviour in the body related to its therapeutic outcome. Factors such as permeability of barriers, pH of varying cellular/body compartments, binding affinity with the off-targets and their fat solubility highly affect the distribution pattern of the natural product in the body. Drug metabolism in the liver or gut introduces alteration in the structure of natural products, as well as irreversible secretion of the drug through hepatobiliary system and or kidney affects the plasma level of the drug and its efficacy. Few reports are available on pharmacokinetics behaviour of natural products (such as glycyrrhetinic acid, curcumin, ethiodized oil) in liver cancer experimental model(s) and or liver cancer patients [103, 104, 134]. The literature revealed that most of the lead anti-liver cancer natural product(s) have not yet been studied for the above discussed pharmacokinetic parameter(s) in the experimental model. Information on the pharmacokinetic parameter of the particular natural products may shed light on the efforts that should be taken to improve their therapeutic efficacy *in vivo* experimental models and liver cancer patients.

New approaches have been practised to improve the natural product delivery and specifically target liver cancer cells. Previously it has been reported that tissue targeted drug delivery significantly enhances the therapeutic efficacy of anti-liver cancer drugs,

confining their bioavailability within the tumor. The concept also minimizes the side-effects such as toxicity by reducing their systemic bioavailability to other organs of the body. Anti-liver cancer drugs combined with a delivery system providing galactose residue have been previously utilized to target liver cells (possess asialoglycoprotein receptors) specifically [135, 136]. Liposomes have been used as a carrier for anti-cancer drug(s) due to various advantages such as improved stability of therapeutic agent(s) in the body without altering the structural integrity of the drug molecule [136, 137]. Recently, *Li et al* studied the effect of natural product encapsulated galactosylated-liposome (NPEGL) to assess its anti-cancer ability and liver cancer cells selectivity potential [138]. The study found that anti-liver cancer activity of the NPEGL was significantly increased comprised to normal natural product-liposome and free natural product treatment in liver cancer cells. Further, the study also reported that enrichment of NPEGL with galactosylated-stearate significantly increased the uptake of the delivery system by the liver cancer cells compared to gastric and non-small cell lung cancer cells [139].

Toxicity due to the off-target effect of the anti-cancer therapeutic drug is also an important problem in the managing liver cancer at the clinical level. It is quite challenging to increase bioavailability and decreasing off-target effects of the anti-liver cancer natural product(s) without compromising its therapeutic efficacy is quite challenging. This situation is more difficult when increased effectiveness of the product is required. Nanotechnology-based approaches are promising to provide the solution to the mentioned problem. It is possible to deliver the natural product(s) using nanotechnology-based strategies, which not only increase the product's biological activity but also enhance its bioavailability. Targeted delivery using these strategies also lowers the elated toxicity issue by reducing the systemic circulation of the product. Recently *Gera et al* synthesized a phytocomposite nanoparticle and studied its anti-cancer efficacy in liver cancer cells. The study reports that natural compound based nanoparticles produced significantly higher anti-proliferation activity in liver cancer cells in comparison to free natural product (non-nano form) [140]. The study suggested

that the increased activity of the nano-formulation of the natural product in comparison to its non-nano form might be attributed to its well dispersed, small-sized particles and thereby increased cellular uptake. The study also suggested that the attraction of the formulation towards the acidic environment of liver cancer cells enhances the output of targeted therapy with lesser or no effect on normal cells. Thus this type of strategy, in combination with other approaches (such as receptor targeting), could be utilized to selectively target the liver cancer cells to avoid the off-target effects and increase the drug's bioavailability [141, 142].

CONCLUSION

In recent years, liver cancer has emerged as a significant public health concern worldwide. Various factors such as viral infection, alcohol abuse, drug-induced liver injury, or a high fat-diet are the leading causes of mortality due to liver diseases. Different signalling pathways, including TGF-β, Wnt/B-catenin, Hedgehog, Notch, EGF, VEGF, Janus kinases and Hippo signaling pathways, are responsible for the progression of liver cancer. First and second-line drug treatments produced better therapeutic outcomes and increased overall disease-free survival in liver cancer patients. Intestinal dysbiosis appears to have a significant role in developing chronic liver diseases. The available modes of treatment include numerous side effects to the human body that could be minimized with the use of natural products such as resveratrol, curcumin, diallyl sulfide and many more. However, natural products-based anti-cancer therapy has also faced some therapeutic limitations, mainly concerning the therapeutic outcome, lower bioavailability, and newer targeted delivery approaches. Targeted drug delivery using encapsulated galactosylated-liposome (NPEGL) and nanoformulations increased the biological activity and bioavailability of the drugs.

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