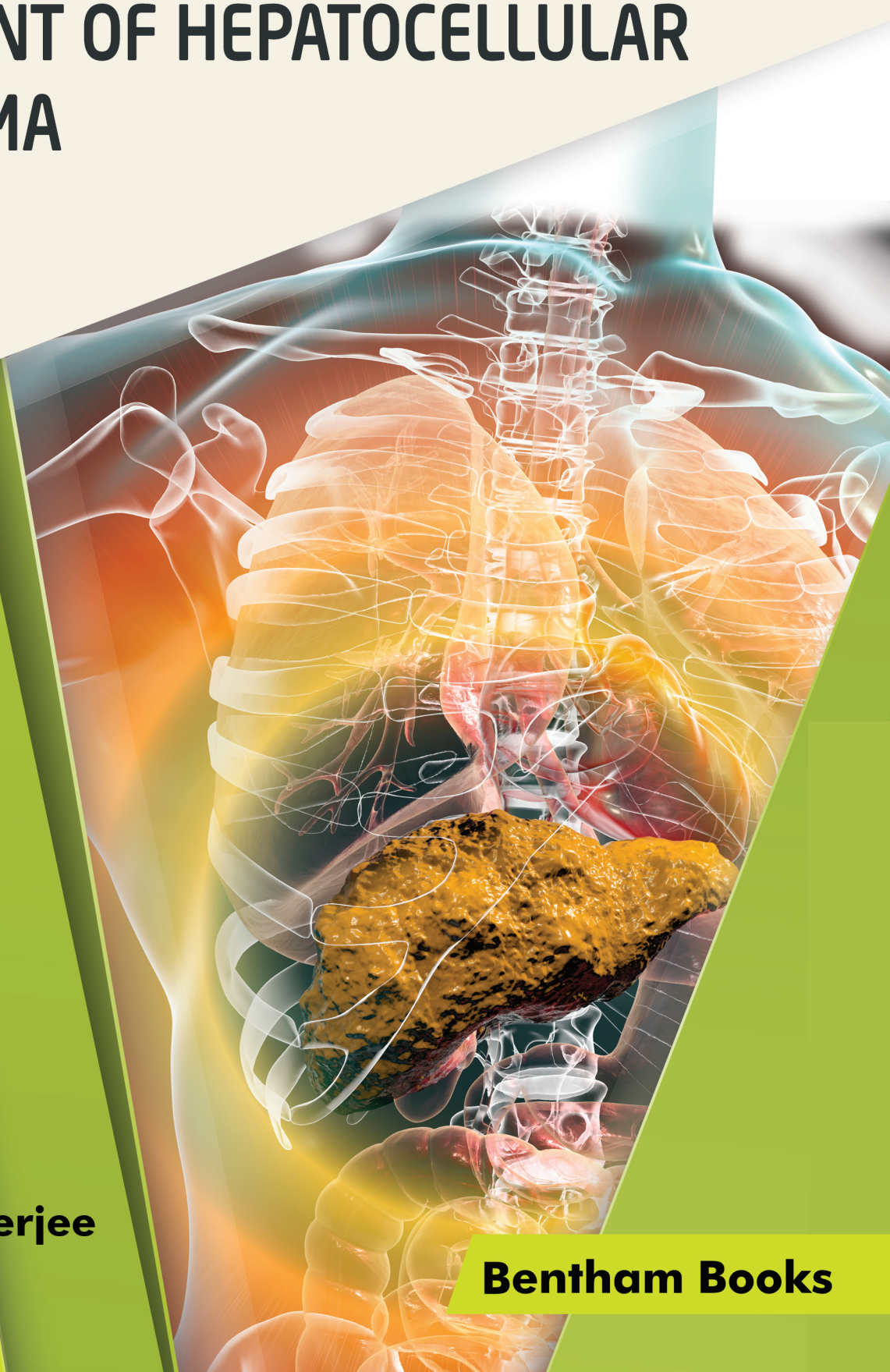
NANOTHERAPEUTICS FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA



Editor:
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Nanotherapeutics for the Treatment of Hepatocellular Carcinoma

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Editor: Biswajit Mukherjee

ISBN (Online): 978-981-5039-74-0

ISBN (Print): 978-981-5039-75-7

ISBN (Paperback): 978-981-5039-76-4

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PREFACE

Cancer is a fatal disease, and hepatocellular carcinoma (HCC) has become a predominant cause of cancer-related deaths. Existing therapy against the HCC is inadequate for several reasons, such as late diagnosis, HCC being asymptomatic at the initial stage, liver resection or transplantation as an advisable treatment option, non-availability of the liver for transplantation, rejection of the transplanted liver by the body, nonspecific chemotherapy that causes damages to cancerous as well as normal hepatic cells, fast rejection of free drug/small molecules from hepatic tumors, the formation of resistance against chemotherapy by cancer cells, and the dearth of the non-targeted drug system.

Hence, it is essential to deliver the drug/formulation specifically to the cancer cells to induce apoptosis and reduction or cessation of proliferation, keeping normal healthy cells unaffected. Nanoscale drug delivery systems show such capabilities for targeted drug delivery to the HCC specific cells, avoiding the normal cells. Several investigations showed how productive and beneficial nanotherapy is in successfully delivering a drug in HCC cells by specifically targeting cancer cells. Commercial availability of the new formulations has been explored and is already in the pipeline of commercialization. Hence, it is important to understand nanotherapy and its correlation to HCC patients to control HCC in a better and satisfactory way. However, technology still needs to be improved for ease of manufacturing and cost-effective production of nanotherapy against HCC. More efforts are required to concentrate on the targeting aspects of the formulation of HCC cells.

The content of this book would be suitable for medical students, medical faculties, researchers of medicines, biomedical scientists, and researchers studying and working in hepatocellular carcinoma and nanotherapy. In this book, several chapters by the authoritative experts of the field have explored all the areas of nanotherapeutics against HCC. The readers will benefit from the knowledge shared by the experts. Thus, nanotherapeutics have shown considerable potential and tremendous impetus for rapid translation from the pre-clinical to the clinical domain to significantly prolong the survival in HCC. Based on the recent investigations, the book is intended to highlight the impact of nanotherapeutics in HCC treatment and its future direction.

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CHAPTER 1

Hepatocellular Carcinoma: Diagnosis, Molecular Pathogenesis, Biomarkers, and Conventional Therapy

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Abstract: Hepatocellular carcinoma (HCC), the most common liver malignancy, has been a significant cause of cancer-related deaths worldwide. Cirrhosis, hepatic viral infections, fatty liver, and alcohol consumption are notable risk factors associated with HCC. Furthermore, a crucial challenge in the therapeutic management of HCC patients is the late-stage diagnosis, primarily due to the asymptomatic early stage. Despite the availability of various preventive techniques, diagnoses, and several treatment options, the mortality rate persists. Ongoing investigation on exploring molecular pathogenesis of HCC and identifying different prognostic and diagnostic markers may intervene in the conventional mode of treatment option for better therapeutic management of the disease. Subsequently, tumor site and its size, extrahepatic spread, and liver function are the underlying fundamental factors in treating treatment modality. The development in both surgical and non-surgical methods has resulted in admirable benefits in the survival rates. Understanding the mechanism(s) of tumor progression and the ability of the tumor cells to develop resistance against drugs is extremely important for designing future therapy concerning HCC. This chapter has accumulated the current literature and provided a vivid description of HCC based on its classification, risk factors, stage-based diagnosis systems, molecular pathogenesis, prognostic/diagnostic markers, and the existing conventional treatment approaches.

Keywords: Cellular signaling pathway, Cirrhosis, HCC molecular pathogenesis, HCC- prognostic/diagnostic markers, HCC risk factors, Hepatocellular carcinoma (HCC), cell signaling during HCC development, Ongoing therapy against HCC, Stage-based diagnosis, Tumor microenvironment.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant form of highly progressive primary liver cancer. It originates from hepatocytes. Around 0.9 million new cases and 0.8 million deaths of liver cancer patients globally have been reported in 2020 by the International Agency for Research on Cancer. Increasing mortality rates and late-stage diagnosis often make HCC a tremendous challenge for its better therapeutic management. Hence patients detected with early-stage HCC possess a greater chance of getting a positive response with different treatment protocols. HCC may not show any symptoms at an early stage of cancer. Still, with the disease's progress, the symptoms such as pain at the right side of the upper abdominal part, fatigue, bloating, loss of appetite, nausea, vomiting, fever, pale bowels, and dark urine may appear. Several risk factors are associated with infected livers, fatty liver, and chronic alcohol consumption in a high amount. In the case of cirrhotic liver, the treatment decisions become limited except the finding of liver transplantation. Due to cirrhosis, any planned liver resection gets limited since the remaining liver may not tolerate volume loss and regenerate. However, an effective treatment method for HCC and cirrhosis of the liver is orthotopic liver transplantation (OLT), but early-stage HCC detection is required for such cases. HCC and cirrhosis are more significant in patients with hepatitis C virus (HCV) infection.

Several upstream or downstream regulators in various signaling cascades activate/ inactivate to continue uncontrolled proliferation in the cancerous processes (Mello and Attardi 2018, Nam and van Deursen 2014, Dolgin 2017). Epigenetic alterations may cause DNA methylation and other histone modifications that confer significant alteration to the genome. The epigenetic modification may inactivate tumor suppressor genes or cause the sudden activation of oncogenes. These may ultimately cause cancer (Kanwal and Gupta, 2012). Thus, a vivid understanding of the tumor microenvironment only can lead to exploring molecular pathogenesis more accurately and minutely to access the more appropriate and convincing therapeutic management of HCC. Suitable diagnostic and prognostic biomarkers are still essential to identify the disease early as the treatment decisions strictly depend on the tumor stage.

Hence, in this chapter, we want to introduce HCC with its classification, risk factors, and various diagnostic staging based on current literature. Tumor microenvironment and molecular pathogenesis during HCC development and progression, along with prognostic/diagnostic HCC markers, have been explored here. The existing conventional treatment approaches give a better understanding of the current way of therapeutic management of HCC.

CANCER AND ITS TYPES

The liver is the largest organ that primarily undergoes detoxification, metabolism, break-down of blood cells, protein synthesis, and bile synthesis. The liver predominantly contains hepatocytes. However, other cell types such as perisinusoidal fat-storing cells or ito cells, hepatic stellate cells, Kupffer cells, and hepatic sinusoidal endothelial cells are also available in the liver (Guyton and Hall 2006, Fox 2011). Neoplasm that grows in epithelial cells is carcinoma, whereas mesenchymal (connective tissue) origin is a sarcoma. Both types appear in the liver.

Primary liver cancer begins in the liver and secondary liver cancer cells where neoplastic cells develop in a different organ and migrate to the liver.

Primary Liver Cancer

Hepatocellular Carcinoma (HCC)

The most common primary liver cancer that accounts for nearly 75 percent of all liver cancers in adults is HCC. It originates from hepatocytes. HCC usually metastasizes to the lungs, bone marrow, and other digestive organs, including the stomach, pancreas, and small and large intestines, including the colon.

Intrahepatic Cholangiocarcinoma (Bile Duct Cancer)

Intrahepatic cholangiocarcinomas originate from epithelial cells of the cell-lining present in small bile ducts. The type accounts for 10-20% of hepatic cancers (Gupta and Dixon 2017).

Angiosarcoma and Hemangiosarcoma

They are primarily rare forms of primary hepatic cancer. Their origin is the endothelial cells of hepatic blood vessels. The reports suggest that these forms of cancer are familiar to people who had prolonged exposure to chemicals such as thorium dioxide and vinyl chloride (Molina and Hernandez 2003, Bolt 2005).

Hepatoblastoma

Unifocal immature fetal precursor liver cells seem to be the origin of this scarce hepatoblastoma. Although rare, hepatoblastoma is only seen in infants and children, usually up to three years of age. Hepatoblastoma can metastasize. (Hoshida *et al.*, 2012, Meyers *et al.*, 2012, Wang *et al.*, 2013).

CHAPTER 2

Hepatocellular Carcinoma and Therapeutic Challenges

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Abstract: Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy and accounts for more than 90% of liver cancers. Despite HBV vaccination programs and targeted therapies, the global burden of HCC is still increasing. Early diagnosis of HCC has been crucial for selecting the best curative treatment options and improving overall patient survival. Despite several advancements in surveillance programs and diagnostic strategies, most HCC cases are still diagnosed at the late stages when most of the current therapeutics become ineffective, making HCC one of the main reasons for cancer-related deaths. Enormous heterogeneity in HCC poses the most prominent challenges for scientists and physicians in designing perfect staging systems and therapeutic selection for HCC patients. Although several HCC therapeutic advancements have come up in the past decade, the current status is far from satisfactory. At present, HCC therapeutics are struggling with several challenges: a shortage of human donors for transplantation, drug resistance, lack of standard operating protocol for immunotherapy, *etc*. Some clinical trials using single or combination therapies are currently underway, hoping to overcome some of these challenges. Constant improvement in HCC therapeutic strategies and prevention measures provides optimism for further advancement in the coming future.

Keywords: Cirrhosis, Early diagnosis, Hepatocellular carcinoma (HCC), Immuno checkpoints, Immunotherapy, Multi-kinase inhibitors (MKIs), Nanoparticle, Prognostic assessments, Systemic therapy, Transplantation.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a significant public health concern and is the most predominant primary liver cancer. Globally, HCC is the 5th most common neoplasm and 3rd leading cause of cancer-related mortality (Wang and Wang, 2019). Worldwide, the numbers of new HCC cases are continuously increasing annually (3-9%) (Velazquez *et al*., 2003). In 2000, the global estimated number of

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HCC cases was 625,000, and due to the high mortality rate of this cancer, the occurrence rate was almost equal to the mortality rate. According to World Health Organization, there will be more than one million liver cancer-related deaths in 2030 (https://www.who.int/healthinfo/global_burden_disease/projections/en/). More than 80% of these HCC cases belong to developing countries, and the male to female ratio is 2.4 to 1 (Parkin, 2001). In 2008, the American Cancer Society estimated 18,000 deaths in HCC in the United States and showed a strong male preponderance (Jemal *et al*., 2007, Siegel *et al*., 2013). HCC patients without any underlying liver disease are sporadic. The major risk factor for the development of HCC is liver cirrhosis, which commonly occurs due to chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), excessive consumption of alcohol, presence of non-alcoholic fatty liver disease (NAFLD), *etc.* (Tinkle and Haas-Kogan, 2012) (Fig. **1**). Studies from different parts of the world have shown that around 75%-80% of HCC cases are due to chronic viral infection (HBV: 50%-55% and HCV: 25%-30%) (Lu *et al*., 2006a, Lu *et al*., 2006b). Due to its higher prevalence rate, HBV outweighs HCV as the most critical risk factor for the development of HCC. A report suggested that the proportion of HCC cases attributable to HBV infection was 16% in the USA, 18% in Western Europe, 51% in Eastern Europe, and 65% in China and the Far East regions (Bharadwaj *et al*., 2013). Other factors associated with HCC development are presence of liver fibrosis, higher viral load, viral genotype (HBV/C; HBV/D1), diabetes, male sex, older-age, exposure to aflatoxins, and a family history of HCC (Datta *et al*., 2018, Datta *et al*., 2014, Yang *et al*., 2011) (Fig. **1**).

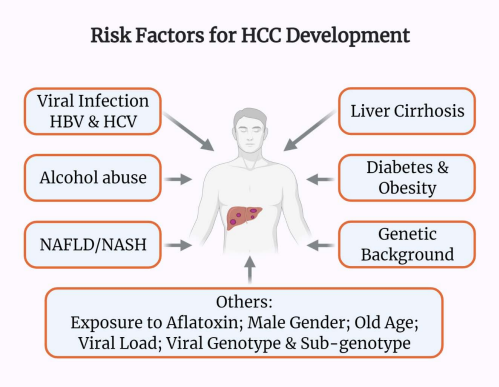

Fig. (1). Schematic diagram showing risk factors for the development of HCC.

The simplest way to prevent HCC-related death is to avoid its risk factors. Nationwide vaccination programs against HBV and broad uses of direct-acting anti-HCV agents are more likely changing the etiologic landscape of HCC (Villanueva, 2019, Forner *et al*., 2018). However, the increasing rate of NAFLD and excessive obesity and metabolic disorders enhance HCC development risk,

imposing a possible future burden of liver cancer in Western countries (Younossi *et al*., 2019). Although the recent CONCORD-3 data published in Lancet showed an increase in survival trends for most cancers because of early diagnosis and treatment, minimal changes were observed in liver cancer survival during a period of 20 years, from 1995 to 2014 (Allemani *et al*., 2018, Anwanwan *et al*., 2020). The present chapter highlights the therapeutic challenges of HCC from diagnosis to therapy precisely.

HCC SURVEILLANCE AND DIAGNOSIS

The development of HCC is a multistep-complex process involving an underlying repetitive cycle of chronic hepatic damage followed by regeneration and repair. Cumulative accumulation of different somatic mutations and epigenetic, transcriptomic, and metabolic alterations make a unique molecular fingerprint for each HCC, explaining enormous clinical and molecular heterogeneity among HCC cases (Marquardt *et al*., 2015, Zucman-Rossi *et al*., 2015). Early-stage diagnosis of HCC is crucial for the selection of proper curative treatments and improved patient survival. Despite several advanced surveillance programs globally, the detection efficiency of early-stage HCC is very low and challenging, mainly because most of the early stages are asymptomatic. Current surveillance imaging techniques are often unaffordable to most patients. Early-stage diagnosis of HCC results in a good prognosis with 5-year survival for more than 70% of patients. In contrast, late-stage diagnosis, which generally happens in most cases, results in less than 16% overall 5-year survival rate (Jemal *et al*., 2007, Siegel *et al*., 2013). In more than 15% of HCC cases, the extrahepatic metastatic spread has been in lungs, lymph nodes, bones, adrenals, and brain at the time of diagnosis, indicating the urgent need to diagnose HCC in the beginning stages (Uka *et al*., 2007, Katyal *et al*., 2000, Choi *et al*., 2009, Yoon *et al*., 2007). According to the American Association for the Study of the Liver Disease (AASLD) guideline, every cirrhotic and high-risk chronic HBV patient should undertake screening with liver ultrasound imaging every six months. Diagnosis of HCC using multi-phase computed tomography (CT) and dynamic magnetic resonance imaging (MRI) is currently going on based on AASLD and the European Association for the Study of the Liver (EASL) guideline. For the detection of HCC, these techniques focused on arterial phase enhancement or vascular shift during malignant transformation of hepatocytes (Bruix *et al*., 2011, European Association for the Study of the Liver 2012, Barr and Hussain, 2014, Willatt *et al*., 2008). This pattern of imaging characteristics has 66-82% sensitivity and more than 90% specificity for the diagnosis of HCC in patients with cirrhotic liver and larger nodule size (>1cm in diameter) (Roberts *et al*., 2018). For patients without cirrhosis and those with no conclusive imaging patterns observed in liver nodules, the diagnosis mainly depends on histologic analysis of liver biopsies.

CHAPTER 3

Nanoformulations and Their Therapeutic Advantages

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Abstract: Cancer therapy involves nanomedicine, which can provide a plethora of advantages unattainable *via* conventional medicine as the materials of nano-level exhibit unique physicochemical and biological properties. Both cancer therapy and cancer therapy research utilize nanoformulations based on liposomes, polymeric nanoparticles, solid lipid nanoparticles, metal nanoparticles, dendrimers, and nanoemulsions for facilitating high specificity negating off-target toxicity, prolonged-release maintaining drug concentration and reducing dosing frequency, increased solubilization and absorption, and penetration of impermeable barriers. The entrée to this chapter is thus made with a brief description of nanomedicine, which is followed by a description of the designing of nanoformulations for therapeutics. Explanations on the types and advantages of nanoformulations are also given. The second section of the chapter describes nanoformulations as therapeutics for cancer, explaining the different targeting strategies and novel approaches involving nanoparticles. Like numerous other cancers, nanoformulations are researched extensively in therapy for hepatocellular carcinoma, the second leading cause of cancer-related deaths. The final section of the chapter deals with the therapeutic advantages of nanoformulations in hepatocellular carcinoma. The prominent nanomaterials investigated in hepatocellular carcinoma therapy include nanoparticles of biopolymers, nanoparticles of artificial biodegradable polymers, metallic nanoparticles, carbon nanotubes, and mesoporous nanoparticles. Targeting of drug-loaded nanoparticles is achieved in therapy for hepatocellular carcinoma *via* passive targeting and/or active targeting. A key milestone in hepatocellular carcinoma therapy is the approval of the drug Zinostatin stimalamer, an emulsion-based formulation, by the Japanese Ministry of Labour, Health, and Welfare.

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Biswajit Mukherjee (Ed.)

Keywords: Active targeting, Bioavailability, Biodegradable, Cancer, Chemotherapy, Controlled release, Dendrimers, Emulsions, Hepatocellular carcinoma, Immunoliposomes, Liposomes, Metal nanoparticles nanoformulations, Nano-medicine, Nanoparticles, Passive targeting, Polymeric nanoparticles, Solid lipid nanoparticles, Therapeutics, Targeted delivery.

INTRODUCTION

Brief Introduction to Nanomedicine

Healthcare and the application of medical practice date back to ancient times and early civilizations. Medicine involves the prevention of illness, restoration of health, and diagnosis and treatment of ailments. When nanotechnology applications are used for diagnosis (nanobiosensors), drug delivery (nanoparticles and nanomaterials), and treatment based on nanodevices, imaging techniques, *etc*. (Zhou *et al*., 2019), that branch of medical applications is called nanomedicine (Karunaratne, 2007). The positive side of nanomedicine deals with the premise that nano-based applications provide a service above the standard conventional methods of medicine. The unique properties, both physicochemical and biological at the nanolevel, have proved nanomedicine to afford conventionally unknown benefits. Thus, many reports based on the nano-level advantages have been documented (Yetisgin *et al*., 2020).

Many of the reported research on nanomedicine are concerned with drug delivery. Therapeutic advantages of nanoformulations are observed when drugs are delivered to the disease site by targeting or improving the bioavailability of the drug to make it more effective. The importance of formulating drugs at the nano level is discussed in the following sections.

Designing Nanoformulations for Therapeutics

In the past decade, nanoformulations of various drugs took center stage with claims for improved delivery and efficacy due to properties at the nano level. Some of the salient features are the ability to target specific tissues (Jafari *et al*., 2012), impart biodegradability, increase bioavailability (Yallapu *et al*., 2015), protect the drug in harsh environments, particularly in the gastrointestinal tract (GIT), and overcome biological barriers (Khan *et al*., 2018).

The design of nanoformulations must consider the mode of delivery, type of drug, and the carrier to be utilized. Oral administration is a widespread and sought-after drug administration route since it is convenient, non-invasive, economical, and independent of specific medical requirements (Khosa *et al*., 2018; Kermanizadeh

et al., 2018). Oral delivery requires nanoformulation to resist the gastric environment and safely deliver the drug cargo to the intended delivery site. Therefore, the formulation must be resilient to the pH changes and the enzymes encountered throughout the GIT. Nanocarriers facilitate the delivery of drug molecules to target sites in the gastrointestinal tract despite the harsh environment (Plapied *et al*., 2011). Encapsulation of drug molecules in nanocarriers protects them from enzymatic and chemical degradation in the GIT, enhances the absorption in the intestine to reach the bloodstream, facilitates targeted delivery, and also controls the release of drug molecules (Reinholz *et al*., 2018). Once the drug-loaded nanocarrier is in the intestine, it either releases drug molecules for intestinal absorption, or the entire particle has to pass several cell barriers to reach the bloodstream and then the targeted cells (Reinholz *et al*., 2018). Crossing intestinal epithelial cells to reach lamina propia and the endothelial cell layer of blood vessels to reach blood vessel lumen are some challenges for nanocarriers to overcome (Reinholz *et al*., 2018). Therefore, the successful delivery of orally administered therapeutic nanocarriers to targeted cells may be affected by the type, size, composition, and surface modifications of the nanoparticle. For instance, the degradation of liposomes in the gastrointestinal tract is facilitated by the presence of bile salts (Rhee and Mansour, 2011). Modifying the liposome surface with polyethylene glycol (PEG) has imparted some resistance to degradation (Rhee and Mansour, 2011). Regarding orally administered polymeric nanoparticles, chitosan has gained popularity as a natural polymeric material that possesses the ability to increase permeability in the intestine by opening tight junctions (Plapied *et al*., 2011). Likewise, drug formulations intended for colon cancer need to avoid the harsh conditions of the stomach and upper intestine by avoiding degradation and absorption until they reach the colon (Banerjee *et al*., 2017).

On the other hand, the efficacy of topical delivery formulations depends on the penetrability of the dermal barrier. *The stratum corneum* of the epidermis is an obstacle for skin administration of drugs since it limits drug permeation (Zhang *et al*., 2013). The nature of the dermal cells and the epidermal cell composition requires special consideration and the polarity of the drug being delivered when selecting the suitable carrier. For optimum drug encapsulation, combined with its full release, both carrier, and drug compatibilities should be considered. In skin cancers and other skin-related diseases, dermal administration of drugs holds higher efficacy than oral and parenteral delivery (Sala *et al*., 2018). Transdermal drug delivery attracts more interest since those drug particles are not subjected to "first-pass" metabolism and can be achieved with less toxic side effects (Sala *et al*., 2018). Designing suitable nanocarriers enhance the permeation of drugs through the intact skin.

CHAPTER 4

Nanoformulations to Limit Challenges of Conventional Therapy Against Hepatocellular Carcinoma – An Overview

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Abstract: The chapter primarily deals with different nano-drug delivery approaches to overcome current HCC chemotherapy challenges. The objective of organizing the various sections in this chapter is to highlight the different nanoformulations that are currently being explored, benefits of nanoformulations over the free drug, different approaches and molecular blocks utilized for preparation on different nanoformulations and their applications in the context of the development of novel drug delivery approaches in HCC. Conventional chemotherapy and its limitations for HCC, different targeting approaches for HCC, need for nanoparticulate approach in HCC, different strategies for delivery of nanoformulations with different targeting approaches, different nanoformulations (liposomes, micelles, hydrogels, *etc.*) that are currently studied in HCC, different molecular blocks under preparation, the current status of clinical trials of different nanoformulations studied for HCC therapy and perspective for futuristic role of nanoformulations in HCC have been discussed in the present chapter.

Keywords: Active targeting, Albumin, Amphiphilic formulations, Biocompatibility, Clinical trials, Combinational nanoformulations, Emulsomes, Hydrogels, Liposomes, Metallic nanoparticles, Micelles, miRNAs, Multidrug therapy, Multikinase inhibitor, Nanoparticles, Passive targeting, Polysaccharides, Site-specific targeting, Sorafenib, Stimuli-responsive nanoparticles, Targeted delivery.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a liver malignancy and the leading cause of most cancer-related deaths. In the past four decades, liver cancer incidences have more than tripled, with approximately 700,000 new cases diagnosed with liver

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Biswajit Mukherjee (Ed.)

cancer every year (Dhanasekaran *et al.,* 2012). As per the GLOBOCAN report, a significant number of liver cancer cases are reported from the less developed countries with higher occurrences of HCC reported in males than females (2.4:1) (Balogh *et al.,* 2016). Various risk factors contribute to HCC: inflammation, viral infections (Hepatitis-B and -C), toxicity due to alcohol and aflatoxins, and other diseases such as non-alcoholic fatty liver disease, diabetes, cirrhosis, and autoimmune hepatitis. Despite advances in preventive methods, screening, and state-of-the-art technologies in diagnosis and treatment, incidence and mortality continue to rise. From the total HCC cases, only 25% are detected within the early stages, as HCC lacks specific symptoms, leading to advanced non-curable stages. From studies on different HCC samples and body fluids, various biomarkers have been reported for the prognosis and evaluation of HCC. Of these, the well-investigated HCC biomarkers are (1) the AFP, its isoform lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3); and (2) des-γ-carboxy prothrombin (DCP). Further research investigations are underway to establish a few more potential molecules as specific biomarkers of HCC, which includes glypican-3 (GPC3), fibroblast growth factor 3/4 (FGF3/4), glutamine synthase (GS), squamous cell carcinoma antigen (SCCA), Golgi protein 73 (GP73), midkine, osteopontin (OPN), cytokeratin 19 (CK19), heat shock protein 70 (HSP70), Annexin A2, micro-RNAs (miRNAs), Long non-coding RNAs (lncRNAs), circulating tumors cells (CTCs), cell-free DNA (cfDNA).

In the prognosis of HCC, genetic signatures play a crucial role and are accounted for among possible disease biomarkers (Chaiteerakij *et al.,* 2015). Epigenetic changes with modified DNA methylation and histone modifications perturb the gene expression, although the sequence remains unchanged. Interestingly, by deciphering the mechanism at a molecular level, a few epigenetic regulators such as *P16*, DLC1 RhoA GTPase activating protein, *RASSF1A*, runt-related transcription factor 3, and suppressor of cytokine signaling one were observed to be involved in HCC pathogenesis (Khan *et al.,* 2017; Umeda *et al.,* 2018). While the discovery of biomarkers is confined to HCC diagnosis, they are also used for drug targeting (epigenetic modifiers) or to study a therapeutic response (Dhanak *et al.,* 2014; Di Costanzo *et al.,* 2014). For example, panobinostat and belinostat are histone deacetylase inhibitors that have shown good therapeutic efficiency in HCC (Pathil *et al.,* 2006; Yeo *et al.,* 2012). In another study, Zhu *et al.* utilized alpha-fetoprotein (AFP), a biomarker for HCC, to evaluate the therapeutic response and efficacy of ramucirumab following treatment (Zhu *et al.,* 2018).

From histological studies, different subtypes and markers of HCC have been identified and classified to place and confirm various stages of HCC for possible treatment or prognosis. It has been observed that the disease progression shows different developmental patterns (a) A traditional type where the cancer cells

spread tentacle-like growths through the liver (b) a proliferating single tumor can metastasize to other parts of the liver as the disease advances (c) as nodules at several places in the liver are observed in cirrhosis condition, a chronic liver disease (Castaldo *et al.,* 2000). In some cases, the pattern is still non-elucidative as there is no substantial evidence that either correlates with the cause of the disease, cell type being affected, *etc.* Among them, fibrolamellar is a vital indicator that is very uncommon and accumulates up to less than 1% of HCCs, which is most often recognized in younger patients between 10-35 years of age (Lafaro *et al*., 2015). The alarming growth rate of HCC incidence and survival in the population is a grave concern worldwide and requires more attention. While different treatment options are available depending on the stage of HCC profiles such as surgical resection, transarterial embolization (TACE), liver transplantation, radiotherapy, ablation, and chemotherapy, nevertheless, these conventional therapies suffer challenges due to chemoresistance, untoward effects, and promotion of cancer metastasis following surgical intervention (Daher *et al.,* 2018). Most drugs available for treating HCC are conventionally used drugs that exhibit profound cytotoxicity by acting on the vital pathways involving DNA and/or RNA machinery of the cell, arresting its ways for replication, repair, and proliferation (Marin *et al.,* 2020). Thus, prognosis, diagnosis, and therapy of HCC are still not considered successful, and efforts are to utilize the advantages of nanotechnology to manage the disease better therapeutically. The present chapter will focus on nano-drug delivery systems that could limit conventional cancer therapy challenges.

Anticancer Drugs in HCC and its Limitations

Table **1** summarizes a list of conventional anti-cancer drugs with their cellular targets. While these drugs show significant cytotoxicity towards cancerous cells, nevertheless, these are non-selective. These chemicals significantly kill the normal cells leading to severe side/adverse effects limiting its further usage for cancer therapy. The non-specific activity of these drugs arises primarily due to non-uniform bio-distribution of the drug other than the unintended organ, action on multiple biomolecular targets, or activity on a target that has no causative role (Ul-Islam *et al.,* 2018). Table **2** summarizes the adverse effects observed in clinical trials for chemotherapeutic agents intended to treat HCC conditions.

As HCC therapy involves long-term drug therapy, it is vital to develop modalities that can reduce its distribution to other organs, reduce side effects, and improve efficacy. Understandably, the liver being the largest reticuloendothelial organ, researchers worldwide have been focusing on developing specific inhibitors that target the receptors of the causative pathways.

CHAPTER 5

Nanotherapeutics for the Treatment of Hepatocellular Carcinoma

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Abstract: Currently, hepatocellular carcinoma (HCC) is the third leading cause of mortality among cancerous diseases. It is a primary type of liver cancer possessing unique features like solid malignant tumor type growth, leaky vasculature, and angiogenesis. The success of conventional treatment in the management of HCC is constrained due to unresponsiveness to particular approaches, drug resistance, systemic side effects, and recurrence of malignancy. The development of nanotherapeutics offers an impending key for overcoming these challenges. Nanotherapeutics utilizes nanosized or nanostructured materials to attain particular therapeutic and pharmacokinetic purposes. The diverse targeting strategies and site-specific drug release patterns of this approach enlighten the hope for effective management of HCC. Scientists have developed several nanomaterials like nanoparticles, nanogel, and liposomes to deliver chemotherapeutic agents specifically to HCC sites with improved efficacy, safety, and selectivity. Active targeting has remained most common and effective in HCC management among active, passive, and stimuli-responsive targeting strategies. Hopefully, some nanoformulations for HCC treatment have proved their promising effects in clinical trials. In this chapter, an attempt is made to illustrate the overview of HCC, the impact of nanotherapeutics, along with recent developments, suitability, and challenges of various nanotherapeutic approaches for HCC management.

Keywords: Chemotherapy, Hepatocellular carcinoma, Nanomedicine, Nanoparticles, Primary cancer, Nanotheranostic, Selectivity, Targeting.

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INTRODUCTION

Hepatocellular carcinoma (HCC), the most predominant form of liver cancer, is recognized as the 5th common cancer and the third leading cause of mortality among all neoplastic diseases worldwide. It is a primary malignant tumor that occurs as a result of oncogenesis in hepatocytes triggered by multiple risk factors, involving hepatitis B and C viral infections, non-alcoholic fatty liver disease, metabolic syndrome triggered steatohepatitis, chronic smoking, and consumptions of alcohol, aflatoxin B_1 and anabolic steroids (Elnaggar *et al*., 2019).

Several methods, including resection, radiation therapy, thermal ablation, and chemotherapy, are developed for HCC treatment. Yet, the success of the recovery is still a challenge for healthcare professionals as well as patients. Choosing a suitable treatment method for a patient is not easy because of the need to consider the stage of HCC, the magnitude of hepatic damage, the short survival period of patients (usually 1-3 months), and treatment costs. Surgical resection and chemotherapy are the most adopted techniques that have been effective for optimal recovery. Due to the high price, metastatic stage of HCC, and poor liver functionality, more than 80% of patients are unresponsive to surgery. Moreover, the chemotherapeutic agent lacks selectivity and sensitivity to HCC. Thus, it is associated with poor efficacy as well as undesirable toxicities, multidrug resistance, recurrence, and therapeutic failures (Chi *et al*., 2020).

Cancer nanotherapeutics have been achieving enormous popularity in drug delivery and diagnosis of specific cancerous organs and tissues. This approach allows the delivery of nanostructured or nanosized materials with specific therapeutic and/or targeting purposes to achieve promising objectives, including controlling drug release, enhancing drug stability, accumulating drugs at a specific site, imaging, *etc.* In addition, chemotherapeutic drugs (chemotherapy), DNA or RNA (gene therapy), heat-generating drugs (thermotherapy), radioactive isotopes (radiotherapy), diagnostic chemicals (diagnosis), *etc.*, can be delivered successfully to the cancerous region (Wagner *et al*., 2006). Nanotherapeutics offer an emerging scope for managing HCC because of its salient features that may cause acidosis or decreased pH, hypoxia or lack of oxygen, lack of nutrition, and leaky vasculature within the solid tumorous mass (Chi *et al*., 2020). Consequently, scientists have addressed numerous nanotherapeutic approaches aiming at HCC management with promising outcomes in experimental, preclinical, and clinical stages of research.

In this chapter, a substantial overview of nanotherapeutics for effective management of HCC has been discussed with the perspective of associated challenges, recent developmental strategies, and future recommendations.

HEPATOCELLULAR CARCINOMA: AN OVERVIEW

HCC is the deadliest and most predominant (about 80-85%) (Elnaggar *et al*., 2019). It is unique and clinically characterized as the primary malignancy of liver cells, which results from a series of genetic and/or epigenetic alterations leading to trigger initiation of oncogenesis. Multiple symptoms are observed in HCC patients, such as upper right abdominal pain, hepatomegaly, splenomegaly, swelling and bloating in the stomach, anorexia, pale yellowish color of skin, urine, and eyes (jaundice like symptoms), *etc.* The common risk factors for the progression of this disease include chronic hepatic inflammations due to viral hepatitis, parasitic infection in the liver, metabolic syndrome triggered steatohepatitis, fatty liver disease, chronic smoking, and consumptions of alcohol, and several chemicals (aflatoxin B_1, diethylnitrosamine, and anabolic steroids, carbon-tetrachloride and thioacetamide). Besides, obesity, diabetes, age, gender, and lifestyle also influence the progression of the disease. Among these risk factors, hepatitis B infection has been found to progress HCC in 50% of patients (Usmani and Mishra, 2017).

Pathogenesis of HCC and Molecular Targets

Several molecular events, such as lack of sensitivity to anti-growth factors, overexpression of growth factors and specific receptors, angiogenesis, invasion, and metastasis, are the collective outcomes of genetic mutation, amplifications, and deletions, as well as deactivation of genetic material, arises in the progression of HCC (Usmani *et al*., 2018). These molecular alterations vary in the HCC stage and etiology. Accountable functional molecules of these pathogenic pathways would be promising targets for developing treatment strategies of HCC.

Considerable alteration of specific tumor suppressor genes is observed predominantly in the pathogenesis of HCC, particularly in an advanced stage, for example, p53 and retinoblastoma. Besides, signal transduction pathways are altered in this disease resulting in the initiation of oncogenesis in hepatocytes. Tyrosine kinase pathway has been observed as the most prominent pathway that comprises RAS/RAF/MEK/ERK, PI_3K/Akt/mTOR, and NF-kB (nuclear factor-kappa B). RAF is a kinase protein responsible for the hepatocellular proliferation, angiogenesis, and apoptosis. Thus, targeting RAF can be an effective strategy to treat HCC. Another signal transduction pathway, namely the WNT/b-catenin pathway, is also altered in this disease, making cancer highly resistant and nonresponsive to conventional therapies. Moreover, there is an overexpression of growth factors like VEGF (vascular endothelial growth factor) and PDGFRβ (platelet-derived growth factor receptor beta) on cell surface resulting angiogenesis by mediating enhancement of vascular permeability (Elnaggar *et al*.,

CHAPTER 6

A Summarized View of Lipid, Polyplex, Inorganic, and Carbon-Based Nanotherapeutics for Hepatocellular Carcinoma Treatment

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Abstract: Liver cancer is one of the primary causes of global cancer deaths after lung cancer and colorectal cancer. In 2021, an estimated 42,230 new liver cancer cases will be diagnosed, and approximately 30 thousand people will die of these cancers in the United States alone. Hepatocellular carcinoma (HCC) alone accounts for nearly 75% of all liver cancers. Early detection of HCC enables multiple treatment choices resulting in improved therapeutic outcomes. Unfortunately, most HCC cases are typically diagnosed at advanced stages, resulting in poor survival. Among various treatment modalities, chemotherapy remains the mainstay, particularly for treating advanced patients. However, the major drawback of conventional chemotherapeutics is the lack of cancer cell selectivity, leading to significant damage to healthy tissues. These challenges can be circumvented with the help of targeted nanotherapeutics containing anticancer drugs. These nanotherapeutics are increasingly favored over their conventional counterparts due to their specific cancer cell targeting with low off-target effects. Therefore, in this book chapter, we focus on different types of nanocarriers to treat hepatocellular carcinoma. Furthermore, current nanotherapeutics in clinical trials and the future perspective of nanomedicine in liver cancer are discussed.

Keywords: Cancer, Carbon nanotubes, Chemotherapeutics, Chronic hepatitis, Cirrhosis, Drug resistance, Enhanced permeability and retention effect, Fullerenes, Hepatocellular carcinoma, Hepatocytes, Inorganic nanoparticles, Liposomes, Metastases, Micelles, Nanoparticles, Nanostructured lipid carriers, Polyplex, Quantum dots, Solid lipid nanoparticles, Targeted drug delivery.

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INTRODUCTION

Liver cancer is the third leading cause of global cancer death (8.3% of the total cancer deaths) after lung cancer (18.0%) and colorectal cancer (9.4%) (Ferlay J 2020). Liver cancer is much more predominant in Southeast Asia and sub-Saharan Africa than in the USA. In many of these countries, it is the most prevalent cancer type. More than 840,000 people are diagnosed with liver cancer each year globally, in addition to 781,000 annual deaths (Bray *et al*., 2018). In 2021, an estimated 42,230 new liver cancer cases will be diagnosed, and approximately 30,230 people will die of these cancers in the United States, according to the American Cancer Society (Siegel *et al*., 2021). More notably, the liver cancer incidence rate has increased more than three times since 1980, while the death rate has more than doubled during this time.

Hepatocellular carcinoma (HCC) alone accounts for nearly 75% of all liver cancers. However, most of these cases are avoidable since majority of the liver cancer risk factors are modifiable. In addition to cirrhosis and chronic liver diseases, excessive alcohol consumption, cigarette smoking, and viral hepatitis (both hepatitis B and hepatitis C) are also the top risk factors of HCC (Crissien and Frenette 2014, Turati *et al*. 2014, Islami *et al*. 2018). Chronic hepatitis C infection is the most critical risk factor and accounts for 1 in 4 liver cancer cases in the USA (Edlin *et al*., 2015). Cirrhosis can also be developed due to hepatitis, resulting in regenerative nodules due to increased hepatocyte proliferation (Balogh *et al*., 2016). Therefore, it is believed that improved treatment and vaccination against hepatitis can prevent almost 50% of all HCC worldwide.

Early detection of HCC enables multiple treatment choices resulting in improved outcomes (van Meer *et al*. 2015). Numerous modalities are available for the detection of HCC, like ultrasound, computed tomography (CT) imaging, and magnetic resonance imaging (MRI) (Gish 2014). Biological markers such as serum α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) levels are also used in diagnosing HCC (Balogh *et al*. 2016, Gish 2014). Unfortunately, most HCC cases are typically diagnosed at advanced stages, resulting in poor survival (median survival varies between 6-20 months) (Golabi *et al*., 2017). Surgical and non-surgical approaches are available to treat HCC. The patients with liver diseases other than cirrhosis undergo surgical resection, which offers the best chances of 5-year survival at 41–74% (Allemann *et al*., 2013). The surgical removal of the tumor is dependent on its location, size, and remaining liver volume post-surgery. Only patients with confined tumors are suitable for surgical resection (Wong and Frenette 2011). Therefore, candidate selection is a vital part of preventing mortality after surgical procedures. Liver transplantation is also another option for these patients with cirrhosis (Mazzaferro *et al*., 1996).

Among various treatment modalities, chemotherapy remains the mainstay, particularly for treating advanced cases. For unresectable HCC, sorafenib has been used as the first-line treatment ("EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma," 2018). Various other therapies were evaluated; however, no superior efficacy to sorafenib was found. The major drawback of conventional chemotherapeutics is the lack of cancer cell selectivity, leading to significant damage to healthy tissues. Therefore, it is vital to develop novel approaches that enable the targeted delivery of chemotherapeutics to the tumor tissues while sparing the healthy tissue (Brannon-Peppas and Blanchette 2004). Furthermore, most chemotherapeutic drugs lack adequate aqueous solubility and desired pharmacokinetic profiles, causing poor drug distribution at the tumor sites (Danhier *et al*., 2010).

These challenges can be circumvented with the help of targeted nanotherapeutics containing anticancer drugs. These nanotherapeutics are increasingly favored over their conventional counterparts due to their specific cancer cell targeting with low off-target effects (Fig. **1**). However, the true potential of these nanotherapeutics can be realized if they can circumvent various biological barriers and deliver therapeutics to the tumor microenvironment (Sriraman *et al*., 2014, Brigger *et al*., 2012, Sanna *et al*., 2014).

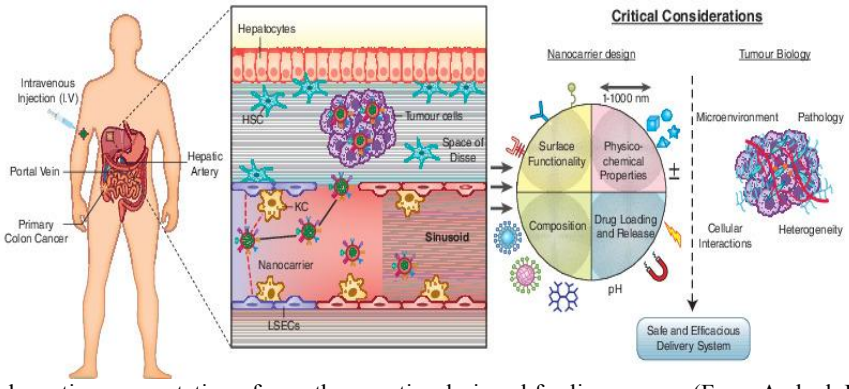

Fig. (1). Schematic representation of nanotherapeutics designed for liver cancer (From Arshad, U, Sutton, PA, Ashford, MB, *et al*. Critical considerations for targeting colorectal liver metastases with nanotechnology. WIREs Nanomed Nanobiotechnol. 2020; 12:e1588, under an open access Creative Commons CC BY license).

The tumor microenvironment contains tumor cells and noncancerous stromal cells, supporting tumor cells in various processes such as proliferation and escaping the immune system (Bissell and Hines 2011). Therefore, for effective treatment, tumor microenvironment modification can be performed by targeting therapeutics to this site (Thakkar *et al*. 2020, Bailey *et al*. 2012). Monumental evidence shows that nanoparticle targeting has led to dose reduction with reduced

CHAPTER 7

Aptamers and Their Potential in Site-Specific Nanotherapy Against Hepatocellular Carcinoma

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Abstract: Globally, hepatocellular carcinoma (HCC) is one of the most devastating neoplasia and has a remarkably high mortality rate. Furthermore, the long latent period associated with HCC lends the diagnosis at the intermediate or advanced stages where the chemotherapy is the solitary therapeutic intervention. The responsiveness of HCC towards conventional chemotherapeutic agents is notably poor due to multiple factors. Among them, multiple drug resistance, reduced drug concentration at the tumor site, quicker clearance, and non-specific distribution are the prime causes leading to remarkably high off-target toxicity and mortality. More importantly, the approval of several multikinase inhibitors (MKIs) by the United States Food and Drug Administration (FDA) for the treatment of HCC as targeted therapeutics has been found to be inadequate to make a notable impact on survival. Therefore, ligand-based targeted therapeutics capable of delivering the therapeutic modality specifically into neoplastic hepatocytes have been explored extensively by researchers worldwide. Among the plethora of HCC-targeting ligands, aptamer-based targeted therapeutics in HCC have gained significant momentum compared to others due to some signature characteristics of aptamer, namely non-immunogenicity, low cost, non-toxicity, thermostability, simpler manufacturing, and high suitability for chemical modification. Despite their enormous potential, aptamer-based targeted therapeutics are still in infancy and require smarter thinking and quick translation from e-clinical to clinical application. Thus, the fundamental focus of the book chapter is to highlight promising features of aptamers, their production, chemical modification, mechanism of action, and finally, detailed emphasis has been given on the overall scenario of aptamer-based targeted therapeutics in HCC.

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Keywords: Aptamers, Diagnosis, Hepatocellular carcinoma, Nanoencapsulated drug delivery, Targeted therapy, Theranostic treatment.

INTRODUCTION

The term 'cancer' can be defined simply as the uncontrolled growth in abnormal cells present in any part of our body. Several types of carcinogens and genetic alterations are considered the primary factors responsible for cancer development (Maitra *et al.,* 2013). The causative agents that can develop normal cells into abnormally behaving cancer cells may include exposure to different chemicals or toxic compounds, pathogenic factors, ionizing radiation, and inherited or acquired mutations in the human genome. Classification of cancer depends on its location in the body and the kind of originating tissue or fluid, although some cancers may be of mixed type. Carcinoma, sarcoma, lymphoma, leukemia, and myeloma are five broad categories of cancer (https://stanfordhealthcare.org/medical-conditions/cancer/cancer/cancertypes.html).

Hepatocellular carcinoma (HCC), commonly characterized as the initial development of malignancy in hepatocytes, is one of the most frequently diagnosed cancer globally but is extraordinarily heterogeneous in nature. Generally, the heterogeneity in HCC is described by rapid dysregulation of cellular signal transduction pathways and the highly unpredictable tumor microenvironment in the neoplastic hepatocytes. Again, these abnormal alterations in the cellular pathophysiological environment are developed through some genetic and epigenetic changes in hepatocytes, and they are very comprehensively associated with multiple metabolic syndromes such as diabetes, obesity or alcohol abuse, non-alcoholic steatohepatitis (NASH) with altered sex, aging, and various environmental factors, including hepatitis B and hepatitis C along with other viral infections and immunogenic disorders (Dagogo-Jack *et al*., 2018). Thus, the therapeutic strategies of HCC are extremely challenging, along with minimum off-target cytotoxicity. Depending on the type and extent of cancer spreading, chemotherapy is chosen to treat cancer. For many years in the past, chemotherapy and radiation were options to save or at least prolong the life span of cancer patients. Radiation delays cancer growth, and on the other hand, chemotherapy helps manage different symptoms caused by cancer. However, the medical community is now aware of the extent of damage the chemotherapy and radiation therapy cause during treatment and the consequent health hazards that follow in the future (https://www.ctoam.com/precision-oncology/why-we-exist/standardtreatment/treatment/chemotherapy/). The need for more sophisti-cated tools for the treatment of cancer will provide selective cell-specific accumulation of chemotherapeutics with reduced side effects to normal cells.

Although chemotherapy has importance in treatment for cancer patients, the toxic effects (*e.g.*, hair loss, nausea, and vomiting, *etc*.) of this conventional cancer therapy that occur due to the inability of chemotherapy to differentiate between cancerous and healthy cells, tendency to develop resistance to the chemical agents, and the necessity of using other treatment forms along with it limit its use (http://www1.udel.edu/chem/C465/senior/fall98/Cancer2/methods.html). Surgery, chemotherapy, and radiation are the mainstream therapeutic approaches for cancer (Padma, 2015; Xu *et al.,* 2001; Dutta *et al.,* 2015; Dutta *et al.,* 2016). The main strategy of targeted therapy is to deliver a drug at the cancer-promoting specific tissue environment or to specific cancerous cells to target a particular protein or genes. The success of the therapy solely depends on targeted drug action in specific cancer cells, thus reducing toxic side effects in normal healthy cells (Gerber *et al.,* 2008; Padma, 2015). By utilizing the "enhanced permeability and retention effects" (EPR) effect, passive targeting to the tumor can be attributed to various macromolecules (Dutta *et al.,* 2019; Kale *et al.,* 2020, Hazra *et al.,* 2021).

On the other hand, direct targeting to a particular target antigen can be achieved through specific monoclonal antibodies or by small molecule drugs that act by altering their signaling. Under indirect approaches, some proteins express specifically on the tumor cell surface and serve as special targeting devices. These can be actively targeted by conjugating the antigen-specific ligand molecules to the drug carrier (Wu *et al.,* 2006).

Different newer technologies have got rapid and huge expansion for the molecular level diagnostics and tumor targeting strategies which made it essential to develop more specific targeting ligands to target definite cell surface molecules expressed uniquely in tumor tissues or cells (Kim *et al.,* 2018). Extensive research is on the pipeline, involving the active targeting to cancer cells utilizing different ligands such as proteins, peptides, monoclonal antibodies, small molecules, and last but not least, the nucleotide sequence-based targeting molecule, aptamers (Ray *et al.,* 2021; Mondal *et al.,* 2019; Mukherjee *et al.,* 2020; Mukherjee *et al.,* 2021). They can recognize cancer cell-specific and unique receptors and bind with them (Sivashankari *et al.,* 2016). The use of aptamer has been widely investigated for its role in targeted therapy, the discovery of biomarkers, *in vivo* imaging, and *in vitro* diagnosis (Sun *et al.,* 2014; Dutta *et al.,* 2018). Aptamers are small, structured oligonucleotides, *i.e*., RNA or single-stranded DNA ligands that are selective and have a high affinity towards the target molecules.

In this chapter, our main objective is to explore aptamer-mediated targeted therapy, its diagnostic and theranostic use, target receptor-specific designing proc-

CHAPTER 8

Preclinical Studies on Nanocarrier-Mediated Delivery of Radiosensitizing Agents to Brain and Pancreatic Cancer with a Future Projection to Liver Cancer

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Abstract: Radiotherapy is one of the primary treatment modalities in oncology. The therapeutic effectiveness of irradiation is dependent on the balance between the tumor control probability (TCP) and the normal tissue complication probability (NTCP), *i.e.*, the induction of side effects. Combination treatment of irradiation with chemotherapy targeted to a tumor or using immune-modulating agents could significantly benefit from nanotechnology strategies, allowing localized delivery of therapeutic compounds to the irradiated tumor volume. When used in combination with irradiation, drugs should be selected on their interaction with the 6 R's, the six Hallmarks of Radiobiology, to sensitize the radiation effect on the molecular, cellular, and tissue level, and in addition to that, positively impact the TCP/NTCP balance. This chapter presents and discusses preclinical data on the combination of irradiation and nanocarrier-mediated delivery of drugs in the brain, pancreatic, and liver cancer. Before implementation into the clinical practice, nanotechnology demands further technical and biological studies on drug loading efficacy, drug release, cellular and tissue uptake, biodistribution, tumor-targeting methods, and administration routes to the patient. Notwithstanding those challenges, the combination of local radiotherapy with tumor-targeted nanocarrier-delivered radiosensitizers, as well as the use of radiosensitizing nanoparticles, are exciting developments with a great clinical prospect.

Keywords: Blood-brain barrier, Binimetinib, Brain tumour, Curcumin, GBM, Hallmarks of radiobiology, Hepatocellular carcinoma, Immune-modulating agents, Nanocarriers, Oncology, Pancreatic cancer, Radiosensitization, Radiotherapy, Six R 's, Therapeutic agents.

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Biswajit Mukherjee (Ed.)

INTRODUCTION

Radiotherapy is one of the most effective treatment modalities in cancer therapy. However, despite modern precision radiotherapy, it is generally unavoidable to deposit ionizing radiation to the tumor volume without risk of radiation injury to the surrounding healthy normal tissues or organs. Hence, the therapeutic effects of radiation are dependent on the balance between tumor control and normal tissue adverse effects. In fact, the tolerance dose of the normal tissues or organs at risk determines the dose which can be applied safely to the tumor volume. For almost all normal tissues and organs, dose-volume constraints are well documented in the literature, the QUANTEC (Quantitative Analyses of Normal Tissue Effects in the Clinic) data, as guidance in the clinical practice (Marks *et al*., 2010). The so-called 'therapeutic window,' the window of opportunity between the tumor control probability (TCP) and normal tissue complication probability (NTCP), is large for some tumor types such as lymphoma but small for other tumor types such as the brain and pancreatic cancer. The main strategy to augment the therapeutic window is to enhance the tumor response *via* combination treatment of irradiation with molecular targeting- or immune-modulating drugs (Begg *et al*., 2011; Bristow *et al*., 2018; Krause *et al*., 2020). Although potential drugs for combination studies should be selected based on their interaction with irradiation, this is still an underexplored field in cancer research (Morris and Harari, 2014). Optimally, drugs should be carefully chosen on their ability to exploit the biological effects of irradiation on cells and tissues, considering the 6R's or Hallmarks of Radiobiology (Fig. **1**) (Section I) (Good and Harrington, 2013; Morris and Harari, 2014).

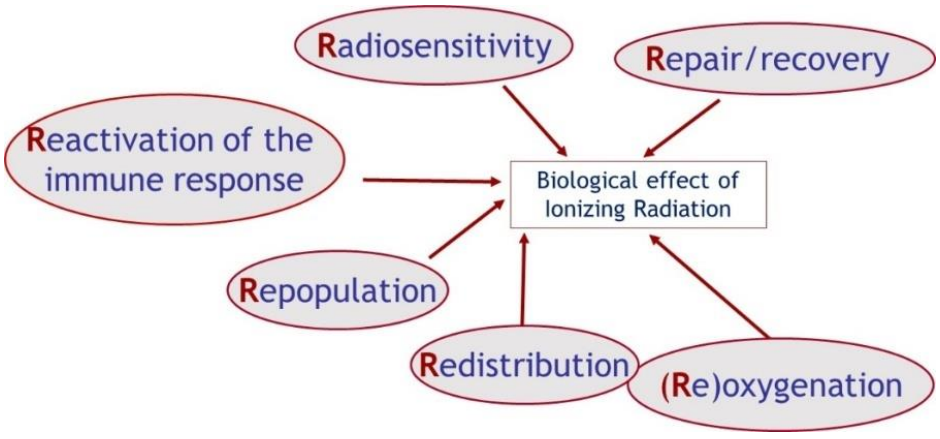

Fig. (1). The Hallmarks of Radiobiology, the 6R's. Six biological phenomena that determine the outcome of radiotherapy in the clinic: *i.e*. the complication rate (side effects due to normal tissue injury) and the tumor control rate (palliation or curation due to tumor sterilization). Details are given in the text.

Section I

Options for modulation of the radiation response should consider these basic principles or Hallmarks of Radiobiology, which has been evolved from Withers' 4R's - 'Recovery/repair, Redistribution, Repopulation and Reoxygenation' - (Withers, 1975), *via* Steels' 5R's - the addition of 'intrinsic cellular Radiosensitivity' - (Steel *et al*., 1989) to 6R's by including 'Reactivation of the immune response' (Boustani *et al*., 2019).

The six Hallmarks of Radiobiology (Fig. **1**) include in brief:

● **Radiosensitivity:** Intrinsic and acquired radioresistance of normal tissue cells and tumour cells, in particular, cancer (stem) cells/the heterogenic cancer cell population.

● **Repair:** Capacity, efficiency, and mechanisms of sublethal DNA damage repair and sensitivity to fractionated irradiation.

● **Redistribution:** The redistribution of cells in the cell cycle affects their radioresistance. Cells in mitosis are most sensitive to radiation, while cells in the S phase are radioresistant. Redistribution following irradiation pushes radioresistant S-phase cells toward a radiosensitive cell cycle phase.

● **Repopulation:** Cell repopulation (not by radiation eradicated cancer cells) is involved in the (accelerated) repopulation of the tumour, as well as repopulation of normal tissue cells to recover from injury.

● **Reoxygenation:** Cells in hypoxic niches within the tumor are radioresistant. Reoxygenation between radiation fractions is an important phenomenon to increase the oxygenation status and therewith tumor cell radiosensitivity.

● **Reactivation of the immune response:** Local irradiation induces systemic immune activation to attack distant tumor cell niches.

Preclinical drug research should, therefore, cautiously identify *radiosensitizing* compounds that enhance the effect of irradiation. In general, the drug dose for radiosensitization is in the lower, non-cytotoxic dose range when given as monotherapy, with the benefit of reduced, if any, systemic toxicity. To be noticed is the close link between the Hallmarks of Radiobiology and the Hallmarks of Cancer and therewith related therapeutic options, which have been discussed in detail (Harrington *et al*., 2007; Bristow *et al*., 2018).

Considering that conventional radiotherapy is generally applied in a scheme with multiple small-sized dose fractions, typically daily fractions of 1.8-2 Gy, five

CHAPTER 9

Preclinical Findings for Targeted Nanotherapies to Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is considered a major ailment throughout the world, and conventional therapies including chemo and combinational have sub-optimal responses with toxicity and adverse effects. The use of conventional methods becomes challenging, especially when the tumor cells adapt resistance rapidly, which further limits their use. Nanotherapeutics for HCC show their potential with minimal toxicity and enhanced degree of targeted drug delivery, which has attracted researchers across the world to explore the various benefits of nanotherapeutics. This chapter has briefly covered the epidemiology and incidence of HCC, its causes, stages, different ways to diagnose HCC, its pathology, and conventional treatment options. We have explained various targeted nanotherapeutic preclinical approaches such as lipidic nanoparticles, polymeric nanoparticles, and liposomes for HCC. Surface-modified nanoparticles and liposomes can actively target a wide array of overexpressed receptors on the tumor surface. It can be seen from the literature that the nanotherapeutic approach for the management of HCC has a high potential to become the mainstream treatment platform if explored and tweaked appropriately. In almost all the works, promising results were seen. Maximum amount of drug was delivered at the tumor site, drug release at unwanted sites were prevented and selectively caused cell necrosis in the tumors, while not affecting the normal cells. These remarkable outcomes further strengthen the nanotherapeutic platform, showcasing its true potential.

Keywords: Asialoglycoprotein receptor, Cisplatin, Cubosomes, Dendrimers, Diosmin, Doxorubicin, Hepatocellular carcinoma, Irinotecan, Liquid crystalline nanoparticles, Liver cancer, Nanoparticles, Nanostructured lipid carriers, Nanotherapy, Polyester vectors, Selenium nanoparticles, Solid lipid nanoparticles, Sorafenib, Smart nanoparticles, Superparamagnetic iron oxide nanoparticles, Tumor suppressor microRNA.

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Biswajit Mukherjee (Ed.)

INTRODUCTION

The liver is the largest and essential organ of our body as it plays a prominent role in the metabolism of many substances, including drugs. It helps in eliminating toxic elements from the human body. Liver physiology is unique from other organs as it has super-impossible networks that are one outlet and two inlets (Lorente *et al*. 2020). Apart from metabolism, the liver also aids in the storage of vitamins, and an important fluid in the bile is also secreted by the liver. The liver also aids in thyroid hormone functioning, where it acts as a place for T3 and T4 de-iodination. The prevalence of liver diseases depends on ethnicity, gender, and socioeconomic status. East and North Africa, Latin America, and Caribbean regions are most prone to liver diseases (Aa *et al*., 2014).

Liver cancer is a type of cancer that affects hepatocytes, and there are three types of liver cancer. Hepatocellular carcinoma (HCC) is the most common type of liver cancer, around 75% of liver cancers belong to this category, and it originates in hepatocytes. Cirrhosis, Hepatitis virus B (HBV), and C (HCV) infections are the main causative reasons for HCC. Cholangiocarcinoma mainly occurs in the tube-like small bile ducts where they carry bile acids to the gallbladder. Around 10 to 20% of hepatocarcinoma arises from Cholangiocarcinoma. Angiosarcoma accounts for about 1% of all types of hepatocarcinoma, and it starts in the blood vessels of the liver and grows fast. Metastatic cancer in the liver develops when primary carcinoma spreads from other part(s) of the body to the liver. Colorectal cancer causes most liver metastases (Types of Liver Cancer: Common, Rare and More Varieties. 2018). HCC is one of the cancers which causes most number of deaths. More than 80% of liver cancers attribute to HCC (El–Serag and Rudolph 2007). Around 85% of cases of HCC are reported in countries like eastern Africa and Asia (Tang *et al*., 2018). Non-alcoholic steatohepatitis (NASH), HBV and HCV infection, liver cirrhosis, excessive alcohol consumption, and ingestion of Aflatoxin and aristolochic acid are important risk factors for developing HCC (Ma *et al*. 2013). This chapter highlighted the findings of targeted nanotherapeutics to hepatocellular carcinoma from some selective preclinical investigations. Further, in brief description of the causes, staging, diagnostic and therapeutic aspects of HCC has been provided.

CAUSES OF HCC

Viral Hepatitis and Cirrhosis

Around 80% of cases of HCC are due to prolonged infection of HBV and HCV globally (Yang and Roberts 2010; Park *et al*. 2015). In developed countries, most of the HCC cases are developed as a continuation of a prolonged chronic type of hepatitis. Annually, 2 to 5% of HCC cases are reported in cirrhosis patients (Yang

et al., 2017a). A Taiwan-based study on 11801 male patients reported an increased chance for the development of HCC. The increased chance was majorly affected by factors like HBV infection, HCV infection, and alcohol consumption at 55.7%, 15.3%, and 2.1%, respectively. This study also reported an association of HBV and HCV infections had enhanced the development of HCC by 1.7%. The combination of HBV infection with alcohol consumption enhances the risk by approximately 4.2% (Liao *et al*., 2012).

Fatty Liver Disease and Diabetes

Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of liver disease development and increases the risk factor of HCC (Younossi *et al*., 2016). A range of 10-20% of the HCC cases in the US is accredited to NAFLD. According to a study, NAFLD was associated with a 2.6-fold increased risk to HCC (Younossi *et al*., 2015). It is also seen that NAFLD-linked HCC frequently occurs even in cases where cirrhosis was absent (Yang *et al*., 2017b). Obesity and diabetes mellitus also cause an increase in NAFLD (Welzel *et al*., 2013). A report suggests a 2 to 3-fold increase in the chances of HCC in patients with diabetes mellitus (Huang *et al*., 2018). Another study reported that diabetes mellitus enhances the chances of HCC in patients with cirrhosis. The resistance of insulin and the production of reactive oxygen species, which triggers the inflammation of the liver, is known to have an important role in hepatocarcinogenesis (Hui *et al*., 2008).

Alcohol

Cirrhosis caused by alcohol is a common risk factor for HCC. World cancer research fund conducted some studies and reported that consumption of 10 g alcohol a day increases the chances of suffering from HCC by 4%. In contrast, when compared with patients who have chronic viral hepatitis, the patients related to alcoholic cirrhosis have fewer chances of developing the HCC (West *et al*., 2017). A retrospective cohort study conducted in a single center comprising 450 alcoholic cirrhosis patients revealed independent risk factors (thrombocytopenia and old age) that led to the development of HCC (Mancebo *et al*., 2013).

Aflatoxin and Aristolochic Acid

Aflatoxins are mycotoxins that have hepatocarcinogenic properties and are adulterated into different kinds of cereal and oilseeds. Ingestion of aflatoxin is one of the risk factors for HCC. Aspergillus sp. produces the aflatoxin B1, which is the main form of the aflatoxins which cause liver carcinogenesis (Wild *et al*. 2015). Mutations at codon 249 in the TP53 tumor suppressor gene caused by Aflatoxin B1 (AGG to AGT) lead to the substitution of arginine for serine

CHAPTER 10

Potential Nanoformulation Approaches for Delivery of HCC Therapeutic Agents

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Abstract: Hepatocellular carcinoma (HCC) is a major cancer type worldwide. The major challenge with HCC is that it is usually detected at an advanced stage. After the launch of the first drug Sorafenib against HCC in 2007, several other small molecules and monoclonal antibody-based drugs have been launched to fight against HCC. However, the survival rate in HCC is still very poor. This indicates that there is still a necessity for more effective therapy or to increase the efficacy of the existing therapy. Nanoformulation approach refers to such formulation approaches where the particle size of individual particles or vesicles in a formulation range between 1-1000 nm. The individual particle or vesicle could be either single drug particles or nanosphere/nanocapsules having drug entrapped in polymer/lipid matrix or drug connected with metal or metal oxide core. The idea of using nanoformulations for drug targeting first came around the 1950s. Since then, countless research groups across the world have advanced the field to the extent that several products based on the nanoformulation approach have been launched on the market. Besides targeting, nanoparticles can also help circumvent biopharmaceutical challenges of drugs, such as dissolution, limited oral bioavailability issue, or formulating "hard to solubilize" types of molecules for parenteral formulation. This chapter will review different nanoformulation approaches that can be potentially applied for HCC therapy, their manufacturing process, and therapeutic benefits. We will review the application of different nanoformulation approaches for HCC specific therapeutic agents.

Keywords: Liposome, Liquid crystalline nanoparticles (LCN), Micelle, Nano lipid carrier (NLC), Nanocrystal, Nanosuspension, Polymeric nanoparticles, Solid lipid nanoparticle (SLN), Targeted therapy.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the dominant type of liver cancer and, based on recent statistics, is the fourth leading cause of cancer-related death worldwide (El-Serag, 2020). Ten years ago, HCC was the ninth leading cause of cancer death (O'Connor *et al*., 2010, Balogh *et al*., 2016). This indicates that despite the progress of science and medicine in the last decade on various fronts, not much progress has been made against the fight with HCC.

In the past decades, scientific research has brought several therapeutic agents against HCC. These are broadly classified under small molecules, monoclonal antibodies (MCA), RNA-based therapeutics (miRNA or siRNA), and peptides. Among them, small molecules and monoclonal antibodies have been approved for HCC by the United States Food and Drug Administration (USFDA or FDA) (Table **1**) and other regulatory agencies. Sorafenib, a protein kinase inhibitor, was the first drug approved for HCC in 2007. Chemically, Sorafenib is a small molecule. After the approval of Sorafenib, it took almost a decade for the second small molecule Regorafenib to be approved for liver cancer.

Besides small molecules, the other classes of drugs that have been approved for HCC are monoclonal antibodies (MCA). Considering the increased number of MCA approved for HCC related to small molecules, it is clearly understood that this class of drugs is in focus for HCC. The major challenge behind HCC therapy is that in the majority of cases, HCC is detected in an advanced stage, when surgical treatments such as resection and transplantation or locoregional treatment such as chemoembolization do not provide much benefit in terms of the overall survival rate of patients (Forner *et al*., 2018). On the other hand, in such advanced stages, the traditional chemotherapies such as 5-fluorouracil, cisplatin, doxorubicin, or gemcitabine also do not show promising outcomes (Chen *et al*., 2019). Moreover, since the liver is already in a compromised state in an advanced stage of HCC, any systemic therapy with a chemotherapeutic agent puts additional stress on the liver since those drugs need to be metabolized by the liver to eliminate them from the body. Because of the toxicity related issues, MCA are favored over standard chemotherapeutic agents since MCA can help the immune system to differentiate cancerous cells from healthy cells and destroy them. Because none of the drugs mentioned in Table **1** provides a complete cure, instead only an increase of the survival rate by a few months to years depending on the stage at detection, a significant need exists to explore new therapies. The development of targeted therapy is one such focus area for HCC, especially for advanced HCC (Chen *et al*., 2019).

In the pharmaceutical area, "Nano" scale particles are defined as particles within the size of 1 – 1000 nm and cover different types of particles depending on their constituent parts and their physical state, which will be covered in more detail in the next sections. Nanoformulations that are formulations comprising "Nano" sized particles are the major types of novel drug delivery systems that can be used for control release, targeted delivery, and increasing therapeutic efficiency. In one of his articles, Professor Kreuter described the historical background of the concept of nanoparticle and drug targeting (Kreuter, 2007). He mentioned that the idea first came to one of the pioneering scientist Paul Ehrlich when he visited Karl Maria von Weber's Opera "Der Freuschütz" in the 1950s. In that opera, the so-called "Freikugeln," made by calling the spirit of the devil, played a central role. This "Freikugeln" particular property always hit the target even if the rifleman did not aim correctly or if the target was out of reach. Paul Ehrlich thought that this idea of targeting could be applied to improve drug delivery significantly. He named the delivery system "Zauberkugeln" or, in English, "Magic Bullet."With the idea of "Zauberkugeln," Paul Ehrlich started the concept of nanoparticle and drug targeting, which was then carried forwarded by several well-known scientists, such as Prof. Peter Paul Speiser from ETH (Swiss Federal Institute of Technology) Zurich, Prof. Patrick Couvreur from Paris, Prof. Robert Gurny from Geneva, Prof. Jörg Kreuter from Frankfurt and many other great scientists across the world (Kreuter, 2007). Since the start in the 1950s, the nanoparticle area has been revolutionized. Around the mid to late 1970s, various research labs across the world started the concept of polymer-drug conjugates or "nano-therapeutics." The three key technologies in the nano-therapeutics area which became successful from the late 1980s till now are PEGylation (polyethylene glycol conjugated drugs or drug carrier), active targeting (by conjugating cell membrane receptor antibodies, peptides, or small molecule cell ligands to the polymer carrier) and the discovery of EPR effect by Hiroshi Maeda in Kumamoto, Japan. EPR effect, or "Enhanced permeation and retention effect," is referred to as the property of nano-scale carrier to get entrapped inside solid tumors because of the leaky vasculature of the fast-growing tumors, which lead to passive targeting of nano-particles to tumors (Hoffman, 2008).

There is also another type of nanoparticles, where the nanosize particles are pure drug particles or nanocrystals. Often such particles are stabilized in suspension or so-called nanosuspension. Nanocrystals or nanosuspensions are used to solve another big challenge of drug discovery: the poor water solubility of compounds. Most of the chemical entities that come out from combinatorial chemistry as a potential therapeutic agent are poorly water-soluble. Therefore, without using advanced formulation techniques, the oral bioavailability of such molecules remains very poor. Because of poor water solubility, formulating those molecules in a parenteral formulation is not always straightforward. One such HCC specific

CHAPTER 11

Recent Patents and Commercially Available Nanotherapeutics on Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) remains a significant reason for cancer-associated deaths. The diagnostic methods for early-stage detection are insufficient, and there are limited treatments available for the late stages. Thus, novel approaches from nanotechnology have gained much attention to overcome the major hurdles in designing nanoscale materials that could be used in both diagnosis and treatments. Recently approved patents showed that progress had been made with nanotechnology in both diagnosis and therapy. The invention of HCC biomarkers, such as alpha-fetoprotein, liver carboxylesterase 1, glypican-3, endoglin, or CD105, has made significant progress in the area of diagnosis. On the other hand, methods for enhancing the sensitivity and specificity of imaging in nuclear magnetic resonance using Ga^{3+} and nanogold Computed Tomography (CT) contrast agents are noteworthy in the accuracy of cancer imaging. Nanodrugs have long-circulating times inside the body while enhancing the bioavailability of these drugs and improving efficacy without higher doses. Polymer-based nanoparticles, liposomal nanoparticles, and magnetic nano-drug vehicles are used in therapeutics to transport drugs like paclitaxel, docetaxel, doxorubicin, and mitomycin. Antisense oligodeoxynucleotides of midkine, phosphoryl N-fatty acyl nucleosides, siRNAs, and polypeptides have all been used. The long period between clinical trials and commercialization of nanotherapeutics and key issues related to clinical development should be addressed by eliminating the regulatory hurdles limiting nanotherapeutics for HCC in the market.

Keywords: Cancer chemotherapy, Cancer nanotechnology, Clinical trial, Commercial nanotherapeutics, Computed tomography, Diagnosis, Doxorubicin,

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Drug delivery, EPR effect, FDA, Hepatocellular carcinoma, Liposomes, Liver cancer, Nanoparticles, Nanotherapeutics, Nanomedicine, Nuclear magnetic resonance, Patents, Treatments.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor and is the third leading cause of cancer-related deaths globally. It is mainly a significant issue in Asian and African countries. Usually, the lifetime of HCC patients is around five years after being diagnosed with HCC. It is basically due to the lack of symptoms at an early stage, and most of the time, the symptoms appear in the middle and late stages. Therefore, treatment is difficult and results in poor curative effects with a very low five-year survival rate (3%-5%).

Liver transplantation or resection is the curative therapeutic approach for HCC. The transplanting in Barcelona Clinic Liver Cancer (BCLC) stages 0, A, and probably B can be implemented, and the tumor resection is only applicable in stage 0. Hence, most of the late-stage tumors would only be treated with local ablation procedures (Laser-induced thermotherapy (LITT), transarterial chemoembolization (TACE)), or systemic palliative treatment (chemotherapy with sorafenib). The initial finding of cancer is thus essential for effective treatment.

The usual diagnosis techniques such as ultrasonography (US), multiphase computerized tomography (CT), and magnetic resonance imaging (MRI) cannot be used to detect the small HCC nodules ≤2 cm with good sensitivity. (Balogh *et al.*, 2016). Hence, the key to an effective treatment for liver cancer is early detection, early diagnosis, and early treatment. They are often called “three early,” the most important in the early diagnosis. Before clinical symptoms are developed or before the cancer is penetrated, it achieves a higher cure rate and decreases the mortality rate. In this context, engineered nanoparticles-based drug delivery systems have recently been developed for cancer therapy and other biomedical applications (Munaweera *et al*., 2015a, Munaweera *et al*., 2015b, Munaweera *et al*., 2014). An ideal controlled drug delivery vehicle should be equipped with factors such as prolonged circulation of drug molecules in the blood, targeting into the tumor sites, response to local stimuli such as pH/temperature, *etc*., and efficient delivery of drugs into the tumor sites (El-Sawy *et al*., 2018).

Nanotechnology-based discoveries play a significant role in HCC diagnosis, therapeutics, and drug delivery, mainly due to the significant drawbacks and limitations in conventional pharmaceutical agents, formulations, and drug delivery systems (Kumari *et al*., 2016, Mulik *et al*., 2016). One of the critical problems in conventional therapeutics for HCC is the non-specific drug delivery

and toxicity of conventional drug formulations. Nanopharmaceuticals have become a promising solution for the issues mentioned earlier, and several drug formulations based on nanotechnology have been developed in the market now. Several properties of these nanotherapeutics make them applicable for delivering active drugs to the target tumor tissue. Small molecule drugs, like most chemotherapies, have very short circulation time inside the body. The incorporation of nanoparticles can overcome this drawback. These nanodrugs can be made to have a long-circulating time inside the body while enhancing the bioavailability of these drugs and thus improving efficacy without the need for higher doses. Further, nanoparticles offer the ability to control the release of the encapsulated payload. In this regard, a high percentage of the trapped drug is released after the nanoparticles have reached their target tissue. This striking controlled release property of nanoparticles can improve the efficacy of the drugs while reducing off-target toxic effects.

Nanoparticles can be preferentially and easily delivered to the HCC tissues and cells by overcoming the shortcomings of conventional treatment and diagnostic approaches (Munaweera *et al.*, 2016). Nanoparticles have the targeting ability to reach the tumor area in tissue, and they invariably control the dose limit of the active drug. Multiple delivery strategies are included in a well-designed nanosystem by paving the way to reach a higher accumulation in a tumor area. Typically, extensive angiogenesis with defective vascular structure can be seen (Kalyane *et al.*, 2019). Further, a damaged lymphatic drainage system can also be seen. Therefore, increased permeability to circulating nanoparticles is provided through vascular networks of HCC. As a result, a higher concentration of nanoparticles resides in HCC tumor interstitial spaces. This phenomenon is called the enhanced permeation and retention (EPR) effect, and it is the basis for achieving passive targeting of most of the nanosystems (Stylianopoulos, 2013) (Fig. **1**).

This book chapter focuses on patents approved for nanoparticles and other nanoformulations developed for the diagnosis and therapy for HCC and those having commercial applications. Tables **1**, **2**, and **3** present a summary of the patents that have been covered in this book chapter.

CHAPTER 12

Future Direction of Nanotherapy in the Management of Hepatocellular Carcinoma

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Abstract: Worldwide, hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths among humans. Several conventional therapies, including surgical and non-surgical methods such as liver transplantation, radiation, and chemotherapy, have been explored to combat this disease and improve the patients' quality of life. However, due to poor diagnosis of the disease, drug toxicity issues, and difficulties related to liver transplantation, scientists search for novel techniques to treat HCC that ensure targeted drug delivery and help in diagnosing the disease. Nanotherapeutics are a new trend in drug discovery and medicine which deals with nano-sized formulations loaded with various types of materials such as drugs, antibodies, aptamers, genes, viruses, *etc*., and targeted delivery. Moreover, controlled release of the materials can be achieved through modifying their external and internal structures as per requirement. Drug delivery through nano theranostics has taken a new turn as the diagnostic tools tagged with the nano-architectures ensure diagnosis and treatment simultaneously. Nanotheranostics have significant application in the identification of cancer progression through continuous monitoring and treatment of cancer. In this review, we will discuss different beneficial effects and applications of nanotherapeutics against HCC. Along with that, different upcoming strategies, such as personalized medicine, layer-by-layer technologies, implant theory, 3D printing technology, nanorobots, nanocrystals, nano-chips, *etc*., will be discussed here, which may pave the path towards successful diagnosis and treatment of HCC to improve the health of the patients.

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Keywords: Diagnosis, Future therapy, Hepatocellular carcinoma, Nanoparticles, Nanotherapeutics, Technology.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary malignancy that starts in the liver and differs from the other "secondary" liver cancers which spread from other organs to the liver. Early diagnosis can cure HCC with the help of transplant or surgery, but in more advanced cases, the cure is quite tricky where better treatment and proper support can increase the life span of the patients (https://www.webmd.com/cancer/hepatocellular-carcinoma#1). Important risk factors that may lead to developing HCC include hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, liver cirrhosis, alcohol consumption in excessive level, non-alcoholic steatohepatitis (NASH), and also by ingestion of aflatoxin B1 (Pimenta *et al.,* 2010, Gomes *et al.,* 2013, Tunissiolli *et al.,* 2017). The asymptomatic nature of this cancer causes the patients to be undiagnosed at early stages. When detected at the terminal stages, the patients suffer from the severe fatality of liver dysfunction. Therefore, the lack of a sophisticated and efficient tool for HCC diagnosis and late diagnosis at the advanced non-curable stage that excludes the patients from definitive surgical resection (Daher *et al.,* 2018) leaves the option to treat the patients with HCC with palliative care only. Thus the fatal HCC case rate increases worldwide (Stewart 2014, Baig *et al.,* 2019).

Further, the unavailability of competent diagnostic tools for early detection and suitable curable treatment options are the primary reasons that increase cancer-related deaths in HCC patients. Researchers have explored new cases of HCC and its high mortality rate. The investigation aims to improve the existing therapy in patients. In the future, more unique treatments that combat the current lacuna in treating HCC may be developed (Tao *et al.,* 2020).

Choice of treatment for HCC depends on the stage of the disease of the individual. The interdisciplinary evaluation that can guide individual treatment profile of HCC patient includes the tumor stage, liver functioning, performance status, comorbidities if any, and center expertise (Wege *et al.,* 2019).

Management of HCC therapy includes loco-regional therapy such as arterial chemoembolization, radiofrequency ablation, yttrium-90 intra-arterial delivery as microspheres, intra-tumor ethanol injection, microwave coagulation, and last but not least, surgical treatment, which includes surgical resection and liver transplantation (Alnajjar and Elsiesy 2015). Currently, in the late stage HCC therapy, sorafenib has also been considered as first-line therapy, although the

incidence of drug resistance for sorafenib is becoming increasingly common (Tang *et al.,* 2020, Daher *et al.,* 2018).

In current research in the therapeutic management of HCC, the commonly associated complications of conventional therapies are being given attention by scientists worldwide. For the last two decades, they have been involved in finding innovative approaches to understand the development of HCC better and tackle it. Nanotechnology is a novel approach that has established its importance in different areas, including medicine, as confirmed by the interdisciplinary field's speedy growth. It offers unique opportunities for improved therapy for cancer and other diseases through different nanoformulations for improved and targeted drugs with reduced systemic toxicity (Gharpure *et al.,* 2015). Advancement in nanotechnology has drawn attention through the targeted delivery of bioactive anticancer molecules (Baig *et al.,* 2019). Though the application is not limited to treating cancer, it also has an improved profile in cancer diagnostics and imaging (Baetke *et al.,* 2015, Baig *et al.,* 2019). In the field of therapeutic approaches to HCC, nanomedicine has emerged as a great promise for management. Anticancer drug-loaded nanoparticles improve the pattern of biodistribution of the loaded drug, thus reducing the cytotoxic effect of the encapsulated potent anticancer drug and ultimately can overcome the problems associated with HCC management successfully (Malla *et al.,* 2020). Researchers are involved in developing nanomedicines with high biocompatibility, stability, degradability, and lower level of immunogenicity and toxicity,which have been recognized as an emerging field of biomedical research. Targeting of HCC cells with nanotherapeutics may be an obvious path for the treatment of HCC because, in comparison to other hepatic cells, HCC cells undergo genetic and phenotypic changes (Sun *et al.,* 2019).

Various nanomaterials include polymeric nanoparticles, metallic nanoparticles, liposomes, quantum dots, dendrimers, micelles, carbon nanotubes, and nanoshells (Elsabahy *et al.,* 2012, Mohamed *et al.,* 2017). By modifying chemistries of the building blocks and for functional optimization, nanoparticles should be designed to improve permeability through biological barriers, cellular uptake, and protection to the loaded drug from degradation and optimization (Mohamed *et al*., 2017). Researchers have designed different types of drug delivery and targeting strategies for nanoparticles, nanoliposomes, nano micelles, *etc*., for effective targeting to HCC cells through both passive and active targeting (Sun *et al.,* 2019). A tumor can be targeted through nanomedicines in passive mode through leaky vasculature of the tumors by involving enhanced permeability and retention (EPR) effect (Sun *et al.,* 2019). In contrast, the nanoparticles' surface can be modified by incorporating conjugating surface legends with specificity to cancer cell surface receptors (Ghosh *et al.,* 2012, Baig *et al.,* 2019).

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Biswajit Mukherjee

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