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MODERN THERAPEUTIC STRATEGIES FOR CLEVER TARGETING AND TREATMENT OF HEPATOCELLULAR CARCINOMA

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ABSTRACT

Hepatocellular carcinoma is known to be one of the most widespread cancers of all time and has led to death in most of its occurrences. Over the past few decades, different types of therapies have been exploited for treatment of this type of cancer. Chemotherapy and radiotherapy are two of the most widely explored and utilized forms of cancer therapy. When a patient is fortunate enough to have the disease discovered and diagnosed at early stages, the cancer is mostly removed through surgical procedures. However, conventional procedures like surgery, chemotherapy and radiation therapy, have been proven to have some shortcomings or limitations including DNA damage of healthy cells through radiation therapy, side effects like vomiting, fatigue and hair loss during chemotherapy and the usual dangers that come with surgical procedures. Various researches have been performed in recent times to improve the targeting and treatment of hepatocellular carcinoma. This aim of this review is to highlight some of these new innovations.

INTRODUCTION

Hepatocellular carcinoma is considered one of the most popular cancers that has led to death in most of its occurrences worldwide.[1-4] Among the other types of liver cancer cases,

hepatocellular carcinoma accounts for 75% of them. This particular type of liver cancer originates from predominant cells, known as hepatocytes, and tissues in the liver. People who have rigorously damaged liver mostly due to alcohol abuse are most likely to develop this type of cancer. In cases where the disease is discovered and diagnosed at early stages, the cancer is mostly removed through surgical procedures.[4] These surgical procedures include hepatectomy and complete liver transplant. Hepatectomy involves removing the cancerous part of the liver. After a period of time, the remaining healthy liver tissue will grow and develop to replace the removed portion. Liver transplant involves replacing the entire cancerous liver with a healthier one from an appropriate donor. The transplant is only considered when the cancer has not metastasized to other organs in the body. Radiation therapy has been widely investigated and utilized for treating hepatocellular carcinoma.[5] This form of therapy engages the utilization of radiation beams of high energy to annihilate cancer cells and decrease the sizes of tumors. There are two ways for irradiating liver cancers; the external beam radiation and the internal beam radiation. In the external beam radiation, an external body part like the belly or abdomen is aimed at with a beam, while in the internal radiation, a catheter is used to inject minute radioactive spheres into the hepatic artery. Radiation subsequently destructs the blood vessel that supplies blood to the liver, known as the hepatic artery. This will then inhibit the amount of blood being supplied to the tumor in the liver. Radiation can also destroy cancer cells through damaging the DNA of cancerous hepatocytes. This leads to a halt in replication and eventual removal of the cancerous cells from the body. Unfortunately, as effective as radiation therapy is, the risk of it affecting adjoining healthy hepatocytes and also causing side effects like, nausea, fatigue and hair loss are common.

Chemotherapy has also been extensively used for the treatment of hepatocellular carcinoma. It is an aggressive form of drug therapy that aims to destroy cancer cells. The anti-cancer drugs are administered intravenously. The biaryl urea drug, sorafenib, had been the only FDA-approved drug for systemic chemotherapy for advanced hepatocellular carcinoma since 2007.[6, 7] Nonetheless, in April of 2017, a fluorinated variation of this drug, known as regorafenib, was ratified to treat patients who had developed a resistance to sorafenib and hence did not respond to treatment.[8, 9] Some patients yet still have resistant interactions to both sorafenib and regorafenib and would need other types of therapy for managing and treating the disease.[10]

Ablation is another type of therapy utilized in treating hepatocellular carcinoma.[11-13] It involves using heat or alcohol, mostly ethanol, to inject the patients to eradicate cancerous hepatocytes. The area for injection is firstly numbed with anesthesia to prevent the patient from feeling pains before the heat or alcohol is injected. This type of treatment therapy is mostly used for patients who are not qualified or suitable for surgical procedures. Cryoablation and radiofrequency ablation are different forms of ablation that have been used in treating hepatocellular carcinoma.

Combination of some of these therapies have also been widely used in treatment in some instances. Radiation therapy can be used to decrease the sizes of tumors in order to make surgical removal easier. Chemotherapy has also been jointly used with radiotherapy for efficient curing in more severe cases. Chemoembolization is a process in which chemotherapeutic drugs are injected directly into the hepatic artery of the liver during surgery.[14] The hepatic artery is then blocked with other compounds to help keep the medications in the liver for a prolonged period to destroy the cancerous hepatocytes. Certain surgical techniques have similarly involved radiotherapy. This is done to avoid irradiating the normal healthy skin tissue while treating the cancerous hepatocytes directly. This procedure is known as intraoperative radiation. External beam radiation is also utilized after surgery, in some cases, to exterminate any lingering cancer cells that may have been persistent post-surgery.

Although the above discussed therapeutic procedures have been effective in the treatment of hepatocellular carcinoma, they have all been proven to exhibit some shortcomings or side effects.[15- 17] In the last decade, research scientists worldwide have worked on and found innovative ways of advancing these therapeutic methods for treating this vicious disease. The aim of this review is to educate researchers about some of these new innovations in the cancer therapy, pharmaceutical and biomedical fields.

MicroRNA Targeting

MicroRNAs (miRNAs) are minute noncoding RNAs which control gene expression by means of binding to the 3’ untranslated areas on mRNAs.[18] When miRNA is not properly regulated it can lead to a myriad of diseases including Alzheimer’s disease[19], some cardiovascular diseases[20] and of course, various types of cancers.[21, 22] Disney and colleagues decided to explore the advancement of precision therapeutics in the treatment of hepatocellular carcinoma.[23] Their research was based on the above- mentioned

characteristics of miRNAs.

First, a confirmation experiment using qRT-PCR was conducted to establish that Neo-N3 absolutely inhibits miR-525 biogenesis in HepG2 cell line. Here, it was found that Neo-N3 decreased the levels of miR-525−3p by ~40% when HepG2 cells are treated with the compound. It was also confirmed that this decrease was due to biogenesis inhibition and not transcriptional inhibition. This was done by determining how Neo-N3 affected levels of pri- and pre- miR-525. It was observed that pri-miR-525 levels increased by approximately 2-fold after treatment with Neo-N3, whereas pre-miR-525 levels decreased by about 40%. This proved that Neo-N3 bound to the Drosha site and caused the inhibition of the process.

The protein ZNF395 is a transcription factor which is also a downstream target for miR-525'. Research done by Pang et al. showed that about 60% of a liver cancer tissue exhibited miR-525 upregulation through the downregulation of ZNF395 and this stimulated migration and invasion.[24] Hence the second step Disney and his colleagues took was to investigate whether the inhibition of miR-525 biogenesis through Neo-N3 was enough to upregulate ZNF395. This was done through Western blotting and it was discovered that when cells were treated with a certain concentration of Neo-N3 compound, ZNF395 levels did in fact increase. It was also discovered that this upregulation of ZNF395 reduced invasion properties in HepG2 cells.

In furtherance of the above, the effect of Neo-N3 on the levels of other miRNAs was investigated and it was realized that miR-155 richness levels were not affected by Neo-N3 application.

Furthermore, to inquire more on the selectivity of Neo-N3 on the miR-525- ZNF395 circuit, Disney et al. investigated if increased levels of pri-miR-525 reduced the influence of the compound and discovered that overexpression of primiR-525 did indeed reduce potency of Neo-N3 and de-repression of ZNF395.

Therefore, results obtained from this particular research group showed that lucid design methods can be made and customized to treat disease-associated RNAs for potential lead in HCC therapeutics.

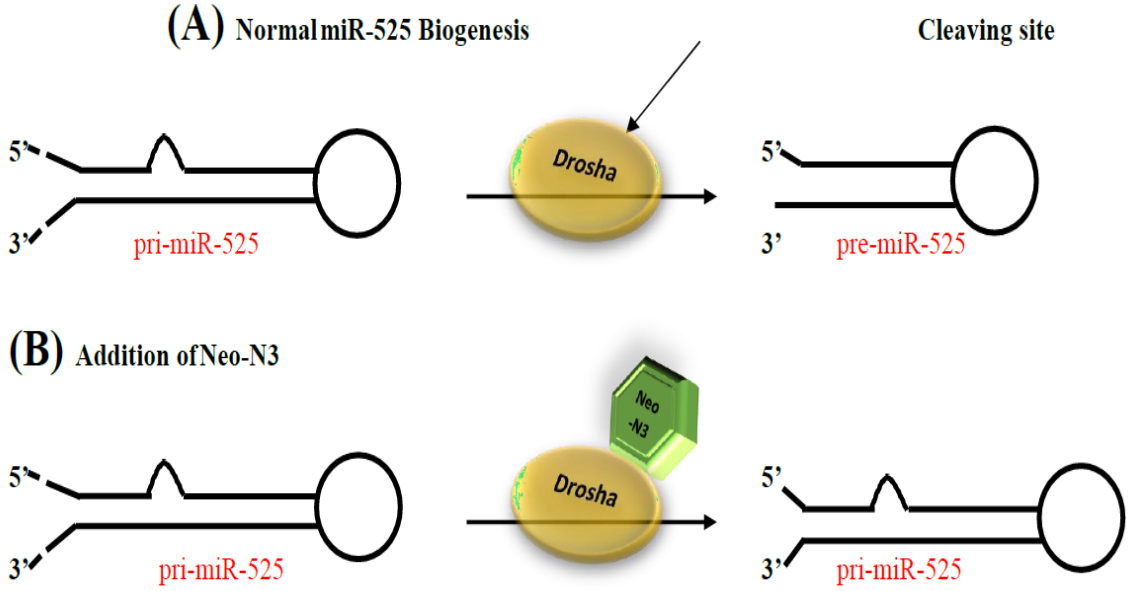

Figure 1: Schematic presentation of how Neo-N3 inhibits miR-525 biogenesis. (A) The function of the enzyme Drosha is to cleave pri-miRNA to form pre-miRNA. (B) Neo-N3 binds to the cleaving site of the Drosha enzyme and hence inhibits the formation of pre-miRNA.[23]

Bonded Pyrimidine-Based Hydroxamates

It has been confirmed through various recent studies, that the activation of the PI3K/Akt/mTOR (phosphatidylinositol 3-kinase-Akt-mammalian target of rapamycin) signaling pathway contributes majorly in HCC growth.[25-29] Class I histone deacetylases (HDAC) have also been found to be abnormally expressed and upregulated in HCC.[25-28] Hence to control these pathways, a series of purine or 5H-pyrrolo [3, 2-d] pyrimidine based hydroxamates were designed by Chen et al., and further advanced as multi-target drugs.[6] The first step of Chen's work involved numerous chemical schematic preparations of the purines and pyrrolo [2, 3-d] pyrimidines sequences through a direct five-step method beginning with modification of commercial 2, 6-dichloropurine or 2, 4-dichloro-7H-pyrrolo [2, 3-d] pyrimidine.

Subsequently, HDAC1 and PI3Kα activities were inhibited. The positive controls used in this step were HDAC inhibitor vorinostat and PI3K inhibitor pictilisib. The cancer cell lines MCF7 (breast cancer), PC-3 (prostate cancer) and MV4-11 (acute monocytic leukemia) were used to assess anti-proliferative activities and variation of targets (HDACs, PI3K, mTOR) in cancer cells.

From previous research done by this particular group of researchers, a purine-based PI3K/mTOR inhibitor with 2-aminopyrimidin-5-yl group was found to be suitable for PI3Kα and mTOR inhibition. It was also realized that the ninth position was susceptible to substituents like rings and chains of rational size.[29] Hence, based on this realization, a series of compounds were first synthesized and used in the inhibition process.

Furthermore, some molecular docking studies using AutoDock Vina software were conducted to further understand the inhibition processes. In vitro profiling against HDACs and kinases as well as anti-proliferation profiling were also performed. Chen and his colleagues then went further to study and evaluate the modulation of HDACs and PI3K/mTOR in cancerous cells and tumors and that of targets in mice bearing tumors. It was then realized after this stage, that among the various pyrimidine-based compounds synthesized, one particular one with a meta-benzyl alcohol group was a potent HDAC inhibitor and was also good for PI3Kα inhibition. This compound, labeled 20f was further used in the next stages of the research.

Clonogenic and soft agar assays, pharmacokinetics and tissue distribution and in vivo antitumor efficacy studies all showed that 20f had effective inhibition properties against HDAC and PI3Kα. After screening of 39 cell lines, the 20f compound was found to be most specific for leukemia, lymphoma and HCC cells. It showed significant efficacy in liver cancer cell lines HuH-7, HepG2 and Hep3B.

As 20f showed outstanding antitumor activity in HepG2 tumor bearing NCr nude mice, further assessments for its dosage−response in female CB17 scid mice were performed. After 26 days, substantial tumor growth inhibition (TGI) with 5/5 regression in 150 mg/kg group (po, QD × 5/week) was realized.

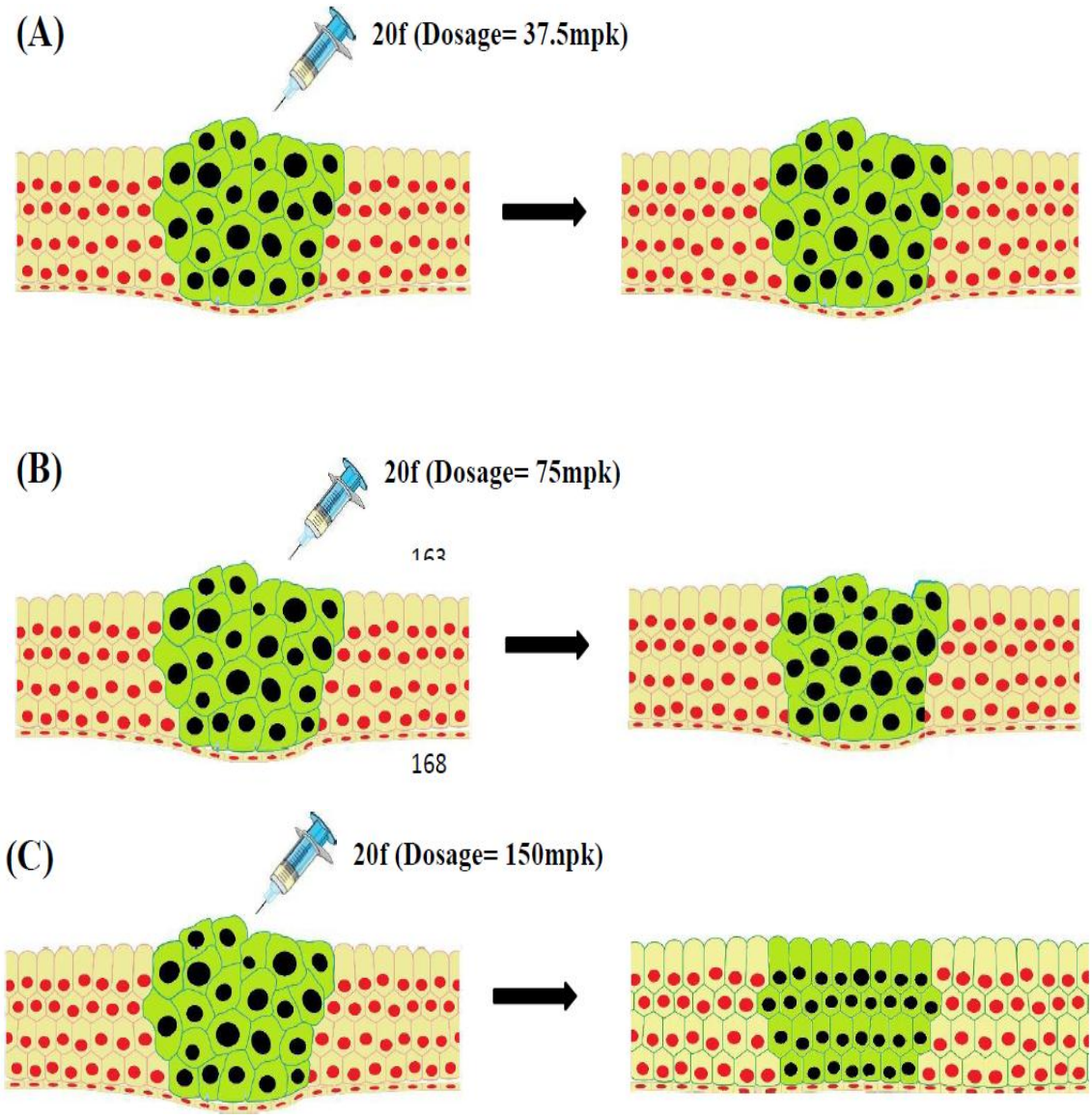

Figure 3: Illustration of dosage−response of 20f (26 days of injections) in female CB17 scid mice. (A) Tumor size remains approximately the same when the dosage is 37.5mg/kg. (B) Tumor size is slightly reduced when dose is increased to 75mg/kg. (C) Tumor size is significantly reduced at the dosage of 150mg/kg.[6]

The results gotten from this particular research group were very significant in the treatment of HCC and therefore there is a need for further development and investigations of the pyrimidine-based hydroxamate with a meta benzyl alcohol group (20f) in liver cancer treatments.

Decrease of Overly-expressed β-catenin target gene- Ornithine Aminotransferase gene Ornithine aminotransferase is a mitochondrial enzyme which is reliant on pyridoxal 5'-phosphate (PLP).[30, 31] The function of this enzyme is to catalyze the interconversion of ornithine and α-

ketoglutarate to L-glutamate semi-aldehyde, which in turn is converted to Δ1-pyrroline-5- carboxylate and glutamate.[16, 30, 31]

Hepatocellular carcinoma has been known to overly express the ornithine aminotransferase gene which is a β-catenin target gene.[32, 33] β-catenin signaling is activated in the liver when the ornithine aminotransferase gene is overexpressed.[34-36] A few investigations have proposed that controlling of the ornithine aminotransferase gene-associated glutamine metabolism by β-catenin is a reliable element for carcinogenesis.[32, 37] This, hence, links the glutamine pathway to liver carcinogenesis.

Zigmond et al. explored the inhibition of ornithine aminotransferase in order to suppress hepatocellular carcinoma.[38] In this research, it was shown that the livers of hepatocellular carcinoma-bearing rats expressed the ornithine aminotransferase gene highly. A gene expression study was conducted on ordinary and spontaneous HCC-developing livers of Psammomys obesus. DNA microarray analysis recognized 7 over-expressed genes and 143 under-expressed genes in the tumor tissues of HCC livers. The OAT gene was identified to be one of the over-expressed genes in the tumors (Figure 4).

For further determination of the role of ornithine aminotransferase in hepatocellular carcinoma development, Zigmond and his colleagues sought for inhibitors. Gabaculine, a natural neurotoxic agent, has been identified to be an effective inhibitory agent of ornithine aminotransferase as well as various other PLP-dependent enzymes via actively binding to PLP to form a stable complex.[39-42] It was, therefore confirmed in Zigmond's research that gabaculine is a concentration, as well as, time-reliant irreversible inhibitor of ornithine aminotransferase. An in vitro analysis on the action of gabaculine on HepA1- 6 and Hep3B cell lines was conducted. After 48 hours of exposing cells to 20000 umol/L of gabaculine, cell proliferation was evaluated with a 3H-thymidine assay. Results showed that gabaculine significantly repressed proliferation, however, the secretion of the HCC biomarker alpha-fetal protein (AFP) was not affected. Nevertheless, when a single dose of the gabaculine was injected into HCC-bearing mice, AFP levels were decreased.

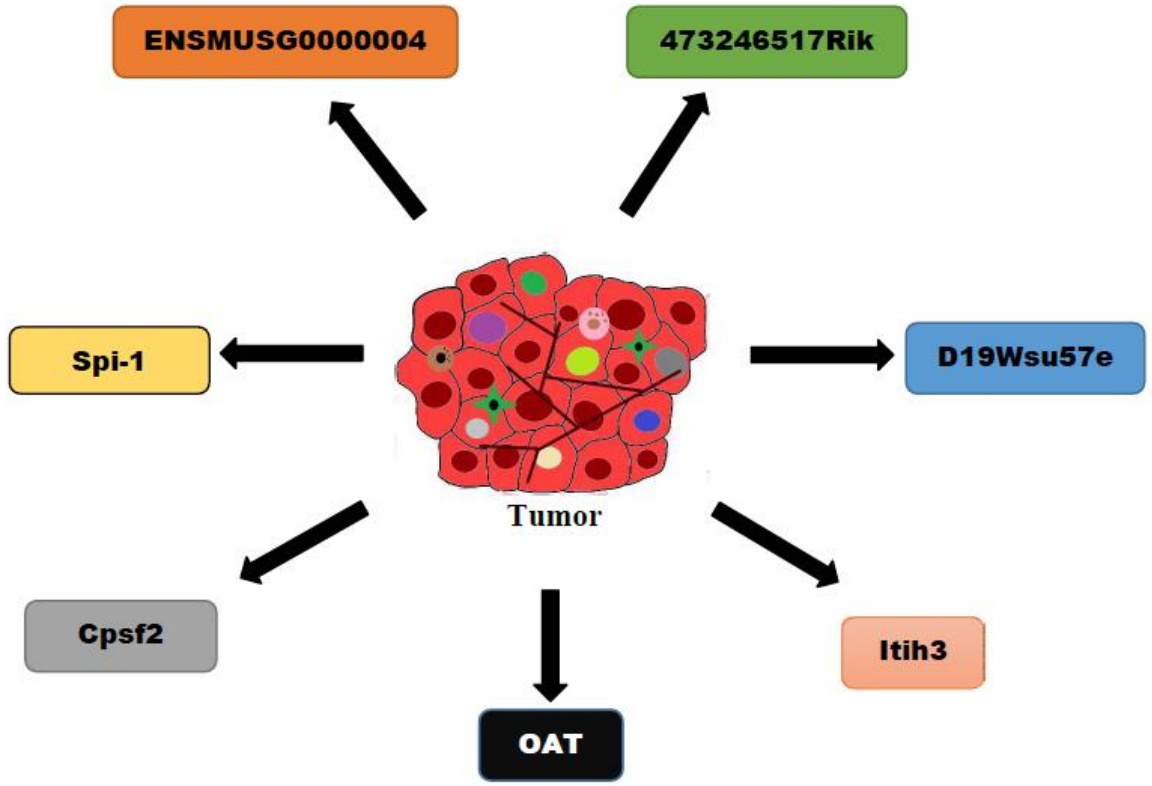

Figure 4: Graphic illustration of 7 over-expressed genes in the tumor tissues of HCC livers. The OAT gene was identified to be one of the over-expressed genes in the tumors.[38]

A library of 24 GABA analogues that were formerly analyzed were screened for compounds that may also selectively inhibit ornithine aminotransferase. (1S, 3S)-3-Amino-4-(hexafluoropropan-2-ylidene) cyclopentane-1-carboxylic acid was found to have significant inhibiting properties. This compound also weakly inhibits L-alanine aminotransferase, L-aspartate aminotransferase and GABA-AT. Significant reduction of AFP levels in both HepG2 and Hep3B cell lines was observed in the in vitro analysis. (1S, 3S)-3-Amino-4-(hexafluoropropan-2-ylidene) cyclopentane-1-carboxylic acid also significantly decreased AFP serum levels and tumor growth in HCC-bearing mice, even at 0.1 mg/kg in the in vivo analysis.

Therefore, it can be concluded that ornithine aminotransferase is a prospective therapeutic target for suppressing the development of hepatocellular carcinoma. Gabaculine and (1S, 3S)-3-Amino-4- (hexafluoropropan-2-ylidene) cyclopentane-1-carboxylic acid are potential inhibitors of this enzymes and hence, should be further exploited for future therapies.

Electro-sprayed Particles Loaded with Gambogic Acid

Gambogic acid is the key active component of the dry resin of the plant species Garcinia hanburyi of the gamboge tree genus.[43] This compound has been found to possess anticancer characteristics and can therefore inhibit the growth of quite a number of different cancerous and non-cancerous tumors.[44,45] Unfortunately, gambogic acid has been found to be highly poisonous to healthy cells and also shows poor aqueous solubility characteristics. This has limited its use in clinical and biomedical applications. Numerous innovations have been explored in the last decade to help improve the anti-cancerous properties of gambogic acid. Researchers have found ways to structurally modify the compound into different variations.[46, 47] This is due to the fact that the 30-carboxy group of the gambogic acid can undergo a variety of modifications while having a slight effect or even no effect on bioactivity. The structural modifications of the compound improved solubility but subsequently product yield was decreased due to the complexity of the synthesis process. Drug delivery systems like magnetic Fe3O4 nanoparticles and polymeric micelles have also been reportedly designed and investigated to help increase the solubility of gambogic acid and also help transport it without harming the healthier cells.[48, 49] However, the entrapment effectiveness is still somehow unsatisfactory and needs to be improved. Yang and co-workers designed a new delivery system to help improve the entrapment efficiency by using electro-sprayed particles.[43]

Electrospray is a highly explored method used to synthesize single-dispersed particles. These particles are characterized by their minute sizes ranging from nanometers to micrometers. The components of an electrospray system constitutes a pump, which serves as the main delivery system, a DC power supply, a grounded electrode, a spray outlet and a material which could be a solution of organic or inorganic or even polymeric materials. The electrospray technique is very simple, cost-effective and has a rapid production period and high entrapment efficiency and retention in activity. The technique has therefore been used to encapsulate biologically active substances like cells, genes, drugs and proteins in particles.[50-53]

The work of Yin and colleagues involved designing and synthesizing a gambogic acid particle delivery system (GA-Ps) using the electrospray technique and used this delivery system to investigate hepatocellular carcinoma treatment.[43] Scanning electron microscope (SEM) analysis of the GA-Ps showed that the particles were smooth and even. The targeting

efficiency of this delivery system was found to be improved. In vivo studies of the antitumor effectiveness showed that GA-P had improved pharmacokinetic features like constancy, retention and clearance time as well as bio-distribution and bioavailabilty. Histological studies of liver performance test through staining showed that GA-P2, which can directly target the liver, presented improved anticancer effectiveness and also showed an inferior extent of liver cancer cell metastasis and invasion. As compared to the GA only, in the GA-P injected murine models, the liver's performance was significantly recuperated and the survival period was also immensely increased in murine models In conclusion, loading electrospray particles with gambogic acid improves anticancer efficacy and seize the shortcomings of just using gambogic by enhancing its solubility in water and also decreasing toxicity on normal healthier cells. Therefore, this form of approach for hepatocellular carcinoma treatment has immense potential and should be further investigated for future applications.

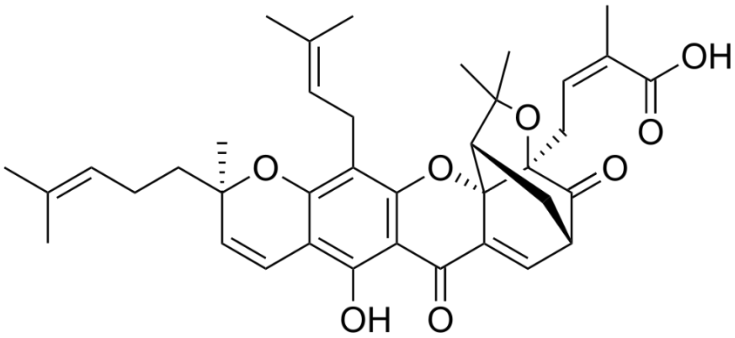

Figure 5: Chemical structure of Gambogic acid.

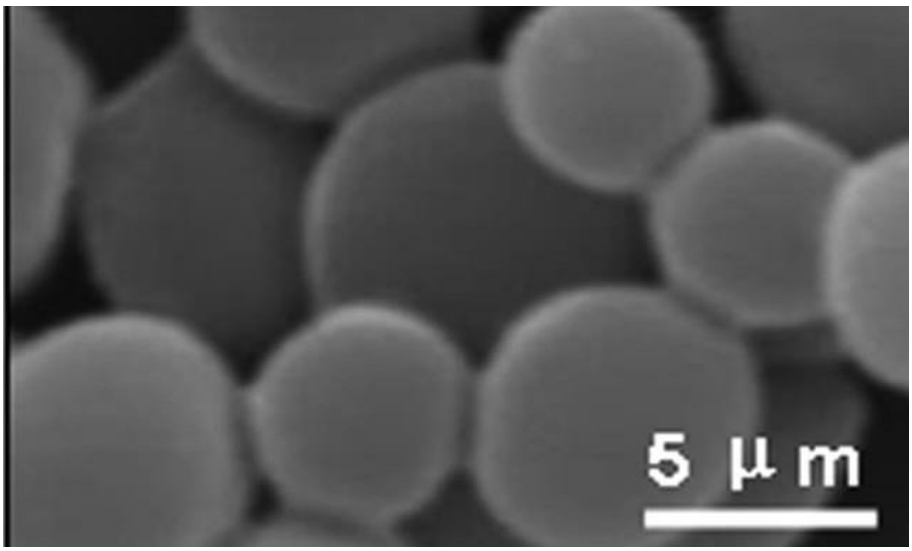

Figure 6: Scanning Electron Microscopy (SEM) imaging of Electrosprayed gambogic acid-loaded particles.[43]

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