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Advances in Experimental and Translational Research in the Treatment of Hepatocellular Carcinoma

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Synopsis

Hepatocellular cancer (HCC) is the fifth-leading cause of cancer and the third-leading cause of cancer related deaths worldwide. Current treatment options are limited as HCC has been shown to be a highly resistant type of cancer to most current treatment modalities. Novel approaches are being explored in the fields of gene therapy, viral oncolytics, radioembolization, and several new biologic therapies. This chapter serves to summarize these recent clinical findings and to discuss what role they will have in the future treatment of HCC.

Introduction

Hepatocellular cancer (HCC) is the most common primary liver malignancy diagnosed worldwide, and its incidence is rising. Currently it is the fifth-leading cause of cancer and the third-leading cause of cancer-related deaths [1,2]. Worldwide, this disease is responsible for approximately 600,000 deaths per year [1]. In the United States due to the increasing incidence of hepatitis C, the incidence of HCC is expected to rise over the next decade [3]. The incidence of HCC doubled in the United States alone from 1975 to 1995 [3,4].

Treatment outcomes for the majority of patients with HCC have remained poor through the years. The overall 5-year survival rate for all patients with HCC has remained steady at 3%–5% [1]. This results from two facts. For most patients, diagnosis is made only when the disease is advanced and inoperable, when the disease has very poor prognosis. Furthermore, the major risk factor for developing HCC is cirrhosis of the liver [5]. In the majority of cases, cirrhosis is caused by chronic infection with hepatitis B or C virus, or by chronic heavy alcohol usage [6,7]. Other identified risk factors include hereditary hemochromatosis, α -1 antitrypsin deficiency, primary biliary cirrhosis, autoimmune hepatitis, smoking, and exposure to aflatoxin (Table 1) [8]. Regardless of the cause, the accompanying cirrhosis can independently cause death as well as complicate other treatments.

Current treatment options for the patient with HCC can be divided into resectional, ablative, and systemic therapies. Resectional therapies include hepatic resection and liver transplantation. Though resection is a potentially curative [9–12], partial hepatectomy can only be offered to a limited number of patients because of technical issues. Liver transplantation as a treatment modality for HCC has demonstrated a vast improvement since

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the establishment of the “Milan Criteria” for the selection of ideal candidates for this procedure. Patients selected according to these guidelines have a 5-year reported survival rate of 50%–75% [9, 13]. However, transplantation is an option only available to a limited number of patients because of limited numbers of available cadaveric livers.

Ablative options, including chemical ablation, thermo ablation, and transarterial therapies, are the most commonly used therapies for HCC. They are based on the slow pace of growth of HCC. Therefore, non curative cytoreduction can produce long term survival [14]. Thermal ablations are now routinely performed for limited numbers of small tumors, while transarterial therapy is standard therapy for large unresectable, or multiple HCCs [15,16]. Ultimately, however, ablative therapies treat only local disease, and most treatments are not curative.

For patients with wide spread metastases, systemic treatment options are limited. In spite of large efforts in preclinical and clinical studies, cytotoxic chemotherapy has limited success. There is no accepted adjuvant or palliative cytotoxic treatment option that has been shown conclusively to prolong survival [17].

Based on the challenges presented by HCC to current and future populations, treatment has been an ever-increasing area of interest for research. Because of disappointing results from cytotoxic therapies, recent efforts have focused on a variety of speculative tactics and techniques. In the subsequent paragraphs we will present some of the most novel areas of current research. We will present preclinical strategies being developed in gene therapy and oncolytic viral therapy. We will then proceed to summarize recent clinical developments in microspheres, radioembolization, and biologic therapies.

Gene Therapy

Gene therapy involves transferring genetic material to a target cell with the aim of correcting a disease. The first clinical protocol utilizing gene therapy was approved in 1990 for the treatment of adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID)[18]. Gene therapy remains a promising approach for the treatment of many diseases, including monogenic diseases, infectious diseases, and cancer. Since 1990, several preclinical and clinical trials involving gene therapy have been conducted, with approximately 60% devoted to cancer treatment [19], including HCC [20].

Gene therapy relies on the ability to induce or transfer genes into target cells, either by viral or physical means. Gene therapy for HCC to date has involved two specific strategies. The first is the prodrug strategy: involving delivery of a gene-directed enzyme that would be able to interact with a nontoxic prodrug to create a local cytotoxic agent. The second is the oncogene suppression strategy, involving either delivery of genes to suppress oncogenes that have been identified in the development of HCC or restoration of native tumor suppressor gene function.

The difficulty of employing these modalities as a treatment for HCC is the ability to design a vector that would be specific for HCC and not the surrounding benign tissue [20]. Several viral vectors, including retroviral, lentiviral, adeno-associated viral (AAV), herpes viral, and adenoviral variations, are currently available for gene therapy and are being explored for HCC gene therapy [21–23]. In addition, Iwasaki *et al.* have recently described the use of nanoparticles as a means of gene delivery. These nanoparticles were engineered to display hepatitis B virus surface L antigen (HBsAg) on their surface [20].

One gene/prodrug combination under study involves the *E. coli* purine nucleoside phosphorylase (*PNP*) gene, which is capable of converting purine analogs into toxic

metabolites. These metabolites are then able to inhibit RNA, protein, and DNA synthesis, leading to cell death [24]. Another gene/prodrug combination being studied is the *Escherichia coli* cytosine deaminase (*CD*) gene with the prodrug 5-fluorocytosine (5-FC). Cells that have been transduced with the *CD* gene will convert the prodrug 5-FC into cytotoxic 5-fluorouracil (5-FU) [24]. This strategy has shown encouraging results in several animal models. Even though 5-FU has only had minor impact on HCC when given as a cytotoxic chemotherapy [25], it is hoped that this gene/prodrug combination might be able to achieve higher local concentrations of 5-FU in effected cells, leading to higher efficacy. These prodrugs are also able to cause a bystander kill effect when activated in one particular cell by producing their desired effect in an adjacent cell.

The prodrug strategy that has been most studied in man for HCC is the herpes simplex virus thymidine kinase (*HSV-tk*) gene given in combination with the prodrug ganciclovir (GCV) (Figure 1). The basis of this strategy is transduction of hepatoma cells with the *HSV-tk* gene. When the well tolerated GCV is subsequently administered, the *HSV-tk* enzyme phosphorylates GCV. Subsequent phosphorylation by cellular kinases produces GCV triphosphates that are incorporated into cellular DNA. Once incorporated, these triphosphates cause inhibition of DNA synthesis, leading to apoptosis of the involved cell [24]. The use of various vectors carrying the *HSV-tk* gene have been reported in the treatment of malignant gliomas, head and neck cancer, colorectal cancer, prostate cancer, and HCC [20]. An additional advantage of the *HSV-tk* gene is that it is also one of the reporter genes that can be used to image tumor transduction by positron emission tomography (PET). Several vectors have been used successfully in imaging of transduced HCC in humans to date [25]. To date, the clinical studies have only demonstrated successful transduction of tumor cells without proof of efficacy.

Another gene therapy strategy under investigation involves the restoration of tumor suppressor genes that have mutated in HCC. The target most studied to date is *p53* [25]. This tumor suppressor gene is responsible for causing cell arrest and the induction of a DNA repair system in response to DNA damage. If the damage sustained by the cell cannot be repaired, it is then signaled for apoptosis. When cells lack a functional *p53* gene, they are unable to stop the cell cycle or trigger apoptosis [19]. One theory has been that the restoration of *p53* will allow the cell to respond more appropriately to apoptotic stimuli. *In vitro* over expression of wild-type *p53* in some HCC cell lines has been shown to induce cell apoptosis or inhibit cell growth regardless of their initial *p53* status [26,27,28]. These positive *in vitro* studies of the restoration of tumor suppressor genes have not been into clinical utility. Part of the worry is that transduction may not be achievable to a sufficient number of cells in vivo to produce clinical efficacy.

An alternative way of achieving suppression of genes important for cancer progression is the use of small inhibitory RNA (siRNA). Such antisense technology is being developed to target the growing list of oncogenes and local growth factors that have been identified in the development of HCC [19,24]. Targets being that have been examined include the *FGF-2*, *IGF-I*, *VEGF-2*, and *COX-2* genes [29–31]. Use of these techniques has shown antitumor effects in vitro and in animal models. This is one of the most promising areas of current research for cancer therapy.

Thus, many targets and manipulations are under preclinical and clinical investigation for cancer and HCC. As our understanding of genetic and proteomic alterations in HCC are improved, increasing numbers of targets are likely to present themselves for molecular-based therapies in this common disease.

Oncolytic Viral therapy—Another novel approach to the treatment of hepatocellular carcinoma is the development of oncolytic viral therapies. This field relies on the natural life cycle of viruses to target and kill cancer cells. The idea is that if viruses are found or engineered to specifically infect and replicate in cancer, then the cell lysis by these viruses can be used as a self-propagating cancer therapy. Evidence that viruses have the propensity for selective infection of cancer has existed for over a century. The first viruses specifically designed for cancer however, did not come about until the last two decades with the work in the 1990s by Martuza *et al.* and Bischoff *et al.* in herpes simplex and adeno- viruses respectively. Since that time a number of clinical trials have involved the use of oncolytic viral agents. A phase I trial from Memorial Sloan-Kettering Cancer Center (MSKCC) produced the first report of oncolytic HSV being injected into the bloodstream of patients with liver metastases [32].

Oncolytic viral therapy relies on the genetic manipulation of wild-type viruses for the purpose of creating a novel virus that will specifically infect, replicate within, and then cause a destructive effect on the malignant cell while leaving the surrounding normal tissue virtually untouched. Different mechanisms are described by which oncolytic viruses are able to destroy tumor cells. Most oncolytic viruses cause direct lysis of the malignant cell as a result of viral replication. Other oncolytic viruses are designed to generate toxins locally at sites of tumor that lead to cell death. Another strategy involves design of oncolytic viruses that either naturally elicit an immune reaction to tumor or secrete artificial immunostimulatory molecules at sites of tumor to promote immune-directed cell killing (Figure 2)[24].

Many molecularly engineered forms of wild-type viruses have been studied as oncolytic agents. Our discussion here will focus on the advances of four different viruses being studied in the treatment of HCC: measles virus, HSV, vesicular stomatitis virus (VSV), and adenovirus.

Measles is a member of the Paramyxoviridae family of viruses. Attenuated strains of the measles virus have been used safely for vaccination since 1963 [33]. One particular strain, the Edmonton vaccine strain (MV-Edm), has been brought to the forefront in research of oncolytic viral therapy. There is abundant preclinical data that this strain of measles can effectively treat animal models of cancer, including multiple myeloma, ovarian cancer, and HCC [34–36]. The oncolytic effect of MV-Edm is promoted by cell-cell fusions caused by the virus. These fusions create a giant cell syncytia, leading to subsequent apoptosis of transformed cells [33]. A recent investigation performed by Blechacz *et al.* described the effects of MV-Edm as an oncolytic agent in the treatment of HCC. They examined the effects of a recombinant MV-Edm that expresses human carcinoembryonic antigen (hCEA). Since few HCC tumors produce hCEA, patient serum levels could theoretically be used as a means of monitoring viral replication. This virus has shown encouraging results in preclinical studies, causing infection, replication within, and causing cell death in the treatment of HCC xenografts [33].

Another promising oncolytic viral agent is the herpes simplex virus (HSV), one of the first viral agents considered for use in oncolytic therapy. Some of the first studies examining HSV in the treatment of cancer were conducted against neurologic tumors. These were performed in order to take advantage of HSVs natural neurotropism. HSV also possesses other characteristics that make it an appealing agent for cancer therapy. Many recombinant versions of HSV have been created and characterized in preclinical studies for a wide spectrum of cancers, including brain, colorectal, lung, gastric, prostate, breast, bladder, and head and neck cancers, and HCC [25,37].

Song *et al.* studied the effects of G207, a replication competent attenuated HSV currently in human brain cancer trials, in the treatment of HCC cell lines. G207 virus is a mutated HSV type 1 with deletions at both $\gamma 34.5$ loci and a disruption of the *ICP6* gene that encodes the large subunit of ribonucleotide reductase. By causing this deletion of the HSV ribonucleotide reductase (RR), G207 selectively replicates in rapidly dividing cells such as malignant cells. The deletion of $\gamma 34.5$ significantly reduces the neurovirulence of the virus [38,39]. In addition, the multiple large mutations in G207 make spontaneous reversion to a wild-type HSV phenotype extremely unlikely. G207 is capable of rapid infect of HCC cell lines, leading to efficient replication within, and lysis HCC [40]. Other HSV strains have also been tested against HCC *in vivo*. G29A is a second-generation replication-conditional HSV-1 mutant that is regulated by the albumin enhancer/promoter. This strain is also an effective treatment for orthotopic HCC in nude mice. Oncolytic viruses have also been investigated as potential vectors for combined oncolytic therapy and gene therapy for HCC. Interleukin (IL)-12 has been genetically engineered into strains of HSV that enable the virus to lyse primary hepatic tumors while producing this immunostimulatory cytokine at the local site of cancer [37].

Another oncolytic viral agent being studied for the treatment of HCC is Vesicular Stomatitis Virus (VSV). This virus has been found to be effective at killing cancer cells that lack the antiviral actions of interferon (IFN) [41]. It is largely nonpathogenic for man because of IFN release in normal cells. This is one of the main properties that make this virus appealing as an oncolytic agent. In animal models, these viruses have proved to be an effective treatment for HCC, with evidence of tumor suppression and in some cases eradication. In animal models of multifocal HCC, reports have shown that VSV delivered via the hepatic artery is most effective [42]. Furthermore, VSV is not endemic to United States, leaving most of the population without preformed immune responses to the virus.

The first adenovirus specifically designed as an oncolytic agent, CI-1042 (ONYX-015), was first described in 1996 [43]. The main mutation performed to create this oncolytic agent involved the deletion of *E1B*. This required the virus to replicate preferentially in malignant cells. Clinical trials indicated that it displayed limited therapeutic effect on HCC. In the past decade additional strains have been developed with strategic promoters and deletions placed in attempts to produce a more efficacious agent. Many of these are currently under clinical investigation [43–45].

One of the main questions that remains unresolved regarding oncolytic viral therapies relates to the host immune response. What will be the effect of this response in the face of repeated dosages for treatment of multifocal HCC, or in a patient previously exposed to the wild-type virus? While considered by some to be a pitfall of this therapeutic option, others postulate that the immune response may act in an additive or even synergistic manner. Oncolytic viral therapy offers great potential in the treatment of HCC, and in treatment of cancer in general. Armed with much encouraging preclinical data, many investigators are seeking clinical data to substantiate use of this class of novel agents as therapy for cancer in man.

Radioembolization—Many other promising treatment modalities for HCC are currently under investigation both in the experimental and clinical phases (Table 2). One of the most promising is the use of radioembolization.

Until now, radiation therapy was not considered a mainstay of HCC treatment [46]. Use of radiation therapy (RT) to treat unresectable liver cancer was limited by the related toxicity. RT delivered to the whole liver produces an entity known as radiation-induced liver disease (RILD), which is characterized by hepatomegaly, ascites, and elevated liver enzymes. This can further lead to complete liver failure in some of the patients. The incidence of

developing RILD is approximately 75% with doses > 40 Gy to the whole liver using conventional external beam radiation treatment [47]. Technological advances in the delivery of external beam RT have been made with the development of 3-dimensional radiation planning techniques. These advanced methods are gaining acceptance but require much expertise for delivery.

The basis for radioembolization, or the intrahepatic delivery of radiation, to treat HCC tumors relies on the hypervascular characteristic of malignant tissue [16]. The hypervascularity of HCC compared with the surrounding benign tissue is well known. The first use of Yttrium-90 microspheres (⁹⁰Y) first began in the 1960s and 1970s with the use of a resin-bound form of the isotope to treat primary and secondary liver malignancies. In the 1980s, radioembolization using ⁹⁰Y encased in glass microspheres was first tested in a canine liver model [16]. This technique involves the injection of radioisotopes that are in micron-sized embolic particles. This modality takes advantage of the tumor vasculature to deliver local radiation. Careful selection of targets is critical. Current contraindications include advanced liver failure, high degree of arterio-venous shunting, flow to the gastrointestinal tract that cannot be corrected using embolization techniques, portal vein thrombosis, and significant extrahepatic disease [14].

Carr describes a phase II trial performed in 2004 in which 65 patients with HCC were treated with radioembolization. Patient comparisons were based in the Okuda Classification system for HCC that was developed in 1985 and is based on tumor size (> or < than 50% of the liver size), serum bilirubin levels (> or < 3mg/dl), serum albumin levels (> or < 3g/dl), and the presence of ascites. The median survival was 649 and 302 days for Okuda I and II patients, respectively, compared with 244 and 64 days, respectively, for historical controls [48]. The Okuda classification system was. Trials such as this have supported the use and investigation of radioembolization as a treatment for liver malignancies.

Biologic Therapies—There is general acceptance that HCC is highly resistant to conventional chemotherapies [8, 49]. In spite of much preclinical and clinical investigative effort, HCC has been found to be a highly resistant type of cancer to a broad spectrum of cytotoxic chemotherapeutic agents, even when given in combination [50]. Until recently, the standard of care was systemic doxorubicin though few responses are noted and little improvement in survival can be expected in the palliative [51], or adjuvant setting [52]. This lack of success of traditional chemotherapeutics has led to active investigation of biologic agents that target cellular proliferative pathways, receptors, and cell surface markers [53]. It is also hoped that toxicity to the patient will also be decreased by more specifically targeting the cancer cell, which is particularly important in patient with underlying cirrhosis.

Such novel biologic therapies have been particularly targeted at growth factors, growth factor receptors, tyrosine kinase pathways, and cell-cell adhesion molecules [49,54]. Different classes of agents such as antibodies and small molecules antagonists have been used to interact at these various points along signal transduction pathways to inhibit tumor growth.

One particularly critical step in the process of HCC tumor development is angiogenesis. The process of blood vessel generation as it relates to tumor invasion and metastasis has been extensively studied. Along the pathways leading to tumor angiogenesis, many potential therapeutic targets have been discovered, such as VEGF, PDGF, IGF, IL-8, FGF, HGF, and nitric oxide [50]. Many of these molecules have been conclusively proven to arise from the tumor or surrounding stroma to participate in the neovascularization of HCC [54]. Angiogenesis is a critical step of in tumorigenesis that can be targeted to interfere with endothelial cells and matrix components. [55, 56]

It was in this area of research that first specifically targeted chemotherapeutics for HCC were evaluated. This first group included the VEGF receptor targeting agents gefitinib, and ceruximab. While these demonstrated initial success their effects are limited suggesting a redundancy in the targeted pathways [49]. PTK, an orally available VEGFR-1 and VEGFR-2 TK inhibitor that has shown promising effects on HCC xenografts in nude mice [57].

This field is already leading to clinical successes. The agent erlotinib (OSI-774), which targets the epidermal growth factor receptor (EGFR) has been definitely demonstrated to improve outcome in patients with unresectable HCC [58]. This agent prevents growth in tumor cells by blocking EGFR tyrosine kinase activity. The drug sorafenib, which targets Raf kinase and receptor tyrosine kinases, has been shown in Phase II clinical trials to improve patient survival as described by Abou-Alfa *et al* [59]. Ongoing trials are attempting to determine if combining this agent with ablative therapies will further improve outcome.

It is likely that ongoing studies in targeted biologic therapies will yield additional effective therapies and combination therapies.

Conclusion—The impact of HCC on world health is great, and is expected to continue to rise. Important and innovative research is being conducted to confront this disease from many aspects. Our increasing understanding of tumorigenesis and progression of cancer is fueling fertile investigation into biologic therapies including antibody, small molecule, and gene therapies. In the meantime, physical means of killing tumor, such as local radiotherapy or oncolytic therapy are being perfected to more grossly attack this deadly disease. These investigations are likely to yield combined nonspecific and specific therapies to improve the outcomes of the more than half million patients who die from this disease yearly.

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Figure 1. HSV-tk/Ganciclovir Combination

Cells that have been transduced with the *HSV-tk* gene are able to phosphorylate the prodrug ganciclovir (GCV) to GCV monophosphate. This is then phosphorylated by guanylate kinase (A) to GCV diphosphate. Further phosphorylation by cellular kinases (B) leads to the production of GCV triphosphate, which is incorporated into cellular DNA (C). The incorporation of GCV triphosphate inhibits DNA synthesis and leads to cell death (D).

Figure 2. Mechanism by which viral agents lead to cell death

Different mechanisms are described by which oncolytic agents are able to cause tumor cell death. Most commonly the virus causes direct cellular lysis as a result of viral replication. Other viruses have been described to create antitumor immunity leading to tumor cell death. Still other viral agents destroy tumor cells through the formation of cellular toxins.

Table 1**Risk Factors for Developing Hepatocellular Carcinoma**

Chronic hepatitis B	Fatty liver
Chronic hepatitis C	Autoimmune hepatitis
Chronic alcohol usage	Family history
Hereditary hemochromatosis	Smoking
α -1 antitrypsin deficiency	Exposure to aflatoxin
primary biliary cirrhosis	Glycogen storage disease

Table 2

Additional therapies for the treatment of HCC

90-Yttrium microspheres
External radiation
Cryoablation
Microwave coagulation therapy
Percutaneous ethanol injection
Radiofrequency ablation
Transarterial chemoembolization
Immunotherapy