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World J Gastroenterol 2005;11(42):6571-6576
World Journal of Gastroenterology ISSN 1007-9327

• *REVIEW* •

Immunological treatment of liver tumors

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Received: 2005-01-22 Accepted: 2005-02-18

Abstract

Although multiple options for the treatment of liver tumors have often been described in the past, including liver resection, radiofrequency ablation with or without hepatic pump insertion, laparoscopic liver resection and the use of chemotherapy, the potential of immunotherapy and gene manipulation is still largely unexplored. Immunological therapy by gene manipulation is based on the interaction between virus-based gene delivery systems and dendritic cells. Using viruses as vectors, it is possible to transduce dendritic cells with genes encoding tumor-associated antigens, thus inducing strong humoral and cellular immunity against the antigens themselves. Both chemotherapy and radiation therapy have the disadvantage of destroying healthy cells, thus causing severe side-effects. We need more precisely targeted therapies capable of killing cancer cells while sparing healthy cells. Our goal is to establish a new treatment for solid liver tumors based on the concept of cytoreduction, and propose an innovative algorithm.

Key words: Liver; Tumors; Surgery; Dendritic cell; Cytoreduction; Immunotherapy; Gene manipulation

Chiriva-Internati M, Grizzi F, Jumper CA, Cobos E, Hermonat PL, Frezza EE. Immunological treatment of liver tumors. *World J Gastroenterol* 2005; 11(42): 6571-6576
http://www.wjgnet.com/1007-9327/11/6571.asp

HEPATIC TUMORS

Although relatively uncommon in Western countries, hepatocellular carcinoma (HCC) is probably the most common solid cancer in the world, with an estimated incidence of at least one million new patients per year[1,2]. The optimal treatment for HCC is surgical excision with a curative intent, but only 5-15% of newly diagnosed patients undergo potentially curative resection[3]. Patients with disease confined to the liver may not be candidates for resection because of multifocal disease, or an inadequate hepatic functional reserve capacity related to co-existent cirrhosis may contraindicate resection. As there are few other curative treatment options for patients with unresectable liver disease, HCC is one of the most lethal human malignancies, with a mortality rate of 94%[4].

The liver is second only to lymph nodes as a site of metastases from other solid cancers[5], and may be the only site of metastatic disease particularly in patients with colorectal adenocarcinoma[6]. However, fewer than 10-15% of patients with liver metastases are candidates for resection for the same reasons as those regarding HCC. The majority of patients with primary or metastatic hepatic malignancies who are not candidates for complete surgical resection therefore require novel treatment modalities to control and potentially cure their disease[2,7].

Cirrhosis may be another variable that places such patients at the highest risk[2]. Patients in class C of the Child-Pugh Classification (Table 1) have the highest mortality and morbidity rate following all treatments, particularly surgical procedures[8,9], and so most centers have shifted away from open liver surgery and are attempting other approaches. The treatment of hepatic tumors in cirrhotic and non-cirrhotic patients is a major decision-making issue for oncologists and surgeons, and the high mortality rate of open liver surgery in cirrhotic patients has spurred physicians to seek new modalities[10].

We here outline the immunological and genetic techniques available for the treatment of liver tumors, and propose a new immunologico-clinical algorithm using immunological therapy to debulk the mass, kill micrometastases, and allow a lower dose of chemotherapy to achieve better cytoreduction.

Table 1 Child-Pugh classification

	A	B	C
Ascites	None	Controlled	Uncontrolled
Bilirubin (mmol/L)	<2.0	2.0-2.5	>3.0
Encephalopathy	None	Minimal	Advanced
PT (s prolonged)	<4.0	4.0-6.0	>6.0
INR	<2.0	2.0-3.0	>3
Albumin (g/L)	>3.5	3.0-3.5	<3.0

WHAT IS THE ROLE OF SURGERY?

Open surgery

Complete surgical resection of primary or secondary liver tumors is the gold standard of surgical therapy[2,8,9], but it has fallen out of favor because of complications related to bleeding and liver failure. Furthermore, the time of the associated hospitalization is not cost-effective in the context of the new health plan insurance capitation systems.

Underlying anatomical and physiological limitations may exclude the use of complete surgical resection but, when complete or partial resection is plausible, the approach of choice is either the traditional open technique (wedge resection, segmentectomy or major lobectomy) or the laparoscopic technique. Laparoscopic liver surgery has become feasible with the improvement in laparoscopic techniques and the development of new and dedicated technologies[9]. There are benefits common to all endoscopic procedures, and the choice of the approach to hepatic resection is usually made by both the surgeon and the patient.

Laparoscopic surgery

The laparoscopic method is useful in oncological therapy, as it allows abdominal exploration and the visualization of the tumor itself. Specimen collection is another key benefit, and can range from a lymph node biopsy in the peritoneum or retro-peritoneum, to scraping the peritoneum in the abdominal wall. Laparoscopy allows direct visualization of the organs and biopsy. The liver is a large organ, and can therefore be visualized quite well, particularly the anterior section, although it is laparoscopically more difficult to visualize the posterior section of the retroperitoneal area of the right lobe. Anatomically, the left side of the liver is not hard to mobilize by dissecting the left triangular ligament and flipping the left side of the liver over the midline, but it is more complicated to achieve the same result on the right side where segments VI and VII (the lateral segments) and segment VIII are harder to visualize posteriorly, and so intraoperative ultrasound has been introduced to improve the visualization of tumors in these segments[9].

Radiofrequency ablation

This is a thermal technique designed to cause localized tumor destruction by heating the tumoral tissue to temperatures of more than 50 °C. The methodology has been previously described by our group[8,11], and has been found to be safe and effective in the treatment of single tumors of <5 cm with curative intent, or the cytoreduction of multiple or larger tumors.

Percutaneous ethanol injection (PEI)

This is usually performed under transabdominal ultrasonographic guidance, and consists of intra-tumorally injecting 5-10 mL of ethanol twice a week. Patient compliance has been a problem because of the number of injections required and the associated pain. As PEI requires multiple treatment sessions and is associated with a high local recurrence rate, it should only be considered in the case of tumors with a diameter of less than 1.5 cm.

Cryosurgery

This has been used to treat patients with unresectable primary and metastatic liver tumors for the last 20 years. Most of the scientific data concerning local tumor recurrences and complications after cryosurgery comes from patients treated for colorectal cancer liver metastases[8].

CLINICAL ALGORITHM FOR SOLID LIVER TUMORS

The pros and cons of liver surgery and the new clinical algorithm used for the treatment of liver tumors will be briefly discussed[8], considering only the patients with Child –Pugh class A or B cirrhosis, because those with advanced liver cirrhosis (Child–Pugh class C) would probably receive no survival benefit and would be at a disproportionately increased risk of interventional therapy. The patients in the two groups will belong to one of the following four categories: (1) Those with stage I, primary liver tumors will be evaluated for liver resection or radiofrequency ablation (RFA); (2) Those with stage II and III primary liver tumors will undergo complete resection, if anatomically possible, or partial resection with RFA, or RFA alone; the patients with vascular invasion will also receive a hepatic arterial pump (HAP); (3) The patients with stage IV primary liver tumors or liver metastases of other than colorectal origin (endocrine, breast) will only be treated with RFA and a HAP; (4) The patients with colorectal metastases will undergo complete resection if possible, or partial resection with RFA, or RFA alone, and all will receive a HAP.

After a median follow-up of 20 mo in patients with unresectable liver disease, the addition of adjuvant HAP therapy to cryoreduction decreased all recurrences from 77% to 49% and decreased liver recurrences from 67% to 38%. This, and other multi-approaches (RFA and HAP therapy) to the treatment of partially resectable or unresectable liver disease, is promising and deserves further investigation.

IMMUNOTHERAPY AND NEOPLASTIC LIVER DISEASE

Most cancer patients are currently treated with some combination of surgery, radiation therapy and

chemotherapy, but both chemo- and radiation-therapy have the disadvantage of destroying healthy cells and this causes severe side effects. The possibility of destroying more cancer cells by increasing the chemotherapeutic dose or radiation exposure is limited by the non-specific organ toxicity of these therapies and the relatively old age of most patients. We therefore need more precisely targeted therapies capable of killing cancer cells while sparing healthy cells.

One possible answer is immunological therapy, which is not only more specific and less toxic, but may also induce memory responses that could yield long-term tumor immunosurveillance and reduce the incidence of relapses, thus increasing long-term disease-free survival. Immunological therapy may be adoptive[10,12] in which case the patients' white blood cells are coupled with a naturally producing growth factor to enhance their cancer fighting capacity, or passive[13], with immunity being acquired as a result of the transfer of antibodies from a healthy donor. However, the possibility of successfully implementing these therapies rests on the existence of tumor-specific antigens, and suitable antigens have been hard to come by because of the complex process required to validate them[14–18].

Immunotherapy refers to any approach aimed at mobilizing or manipulating a patient's immune system to treat or cure disease[19], and immunological therapy by means of gene manipulation is based on the interaction between virus-based gene delivery systems and dendritic cells (DCs). Using viruses as vectors, it is possible to transduce DCs with genes encoding tumor-associated antigens (TAA), thus inducing a robust immune response[20,21].

A number of studies have established the role played by DCs in the immune system, and provided a rationale for using them as natural adjuvants for cancer immunotherapy[20-22]. Previous studies have concentrated on identifying the proliferating progenitors of DCs within the small CD34+ sub-fraction of cells in human blood[23]. These cells can be stimulated by cytokines (particularly by GM-CSF and TNF-alpha) to differentiate into DCs *in vitro* over a period of 1 wk[24]. It has also been more recently found that the combination of GM-CSF and IL-4 facilitates the generation of significantly larger numbers of DCs from monocytes/macrophages, which have equal or greater stimulatory activity in mixed lymphocyte reactions, and a greater capacity to present soluble protein antigens than CD34+ cell-derived DCs[23,24].

Gene manipulation

Gene manipulation transmits new genes/DNA into target cells infected with the viral vector, and has been most widely used to treat genetic diseases. The vector unloads its genetic material containing the therapeutic human gene into the target cell, which is finally restored to its normal state as a result of the generation of a functional protein encoded by the therapeutic gene[24,25]. The technique can be used in cancer to activate self and non-self antigens and enhance T cell responses. Some of the different types of viruses used as gene therapy vectors are listed in Table 2.

There are also various non-viral options for gene delivery. The simplest method is to introduce therapeutic DNA directly into target cells, but its application is limited by the fact that it can only be used with certain tissues and requires large amounts of DNA. Another non-viral approach involves creating a liposome (an artificial lipid sphere with an aqueous core), which is capable of shuttling the therapeutic DNA through the target cell's membrane, and a further delivery system is based on electroporation[25-28].

Problems in applying gene therapy

Whenever a foreign body (antigen, bacteria, *etc.*) enters the human tissue, the immune system is prompted to attack the invader, and so there is a risk of stimulating an immune response and reducing the effectiveness of gene manipulation. Furthermore, the immune system's enhanced response to previously encountered invaders makes it difficult for gene therapy to be repeated.

Viruses are the carriers of choice in most gene therapy studies, but they can give rise to a number of potential problems relating to toxicity, immune and inflammatory responses, gene control, and targeting. The main concern is that, once inside the patients, the viral vector may somehow recover its ability to cause disease, which is why we decided to use virus vectors with little or no replicative capacity, such as adeno-associated viruses (AAV)[29-31].

Viral delivery of antigen genes into dendritic cells

There are various ways of inserting antigen genes and proteins into DCs via protein pulses or viral vector loading[27-32]. Recombinant retroviruses, adenoviruses, and poxviruses can all efficiently transduce DCs[29-31],

Table 2 Commonest viruses used as gene therapy vectors

Retroviruses	Adenoviruses	Adeno-associated virus (AAV)	Herpes virus
8kb, RNA enveloped	35 kb, DNA, non-enveloped	5 kb, single stranded DNA, non-enveloped	61 kb, double-stranded DNA
Activate proto-oncogene by insertional mutagenesis	Episomal, transient	Stable integration, high infectivity	Infect mainly neurons
Cause lymphoma	Highly immunogenic, causing inflammation and anaphylactic shock	Non-pathogenic; requires helper viruses such as Adenoviruses for replication and packaging in mammalian cells.	Cause cold sores or blisters in the genital areas
Inactivation of transgene *in vivo*	One case of death	Long-term expression *in vivo*	Cutaneous skin lesions

but they all have well-known and serious disadvantages. Retroviruses can integrate chromosomally, but any residual contaminating wild-type virus can lead to significant disease and malignancy in the host. Furthermore, as they can also integrate gonadally and alter the germ line, their use may be restricted by the FDA[30,31].

Adenoviruses carry many genes in addition to the transgene, and the viral particle contains several proteins; the delivered antigen gene would therefore be only one of the many genes/proteins and epitopes to which a CTL response would be generated.

Unlike these viruses, AAVs are non-pathogenic, and various studies have shown that they are effective gene delivery vectors for both immortalized tissue culture cells and primary hematopoietic cells[33-36]. The helper-dependent parvovirus AAV can latently infect cells via stable chromosomal integration. Early studies demonstrated that 15-30% of immortalized cells could be latently infected with wild-type AAV, and the AAV genome was chromosomally integrated[21]. After the mapping of AAV genes and their functions[32-34], recombinant AAV virus vectors proved to have a similar capacity in immortalized tissue culture cells[33,34], and the recombinant AAV transduction of primary hematopoietic stem cells was achieved in 1988[35].

We have demonstrated that AAVs can be highly efficiently (>90%) used to transduce antigen genes into primary human monocytes (Mo) and Mo-derived DCs[36,37]. Unlike cells transduced using adenoviruses, retroviruses and other pathogenic viruses, AAV-transduced cells are not usually significant targets of the host immune system[37]. The use of the rAAV-based DC loading of human papillomavirus type 16, E6, and E7 antigen genes leads to robust and rapid antigen-specific, MHC class I-restricted CTL responses with one stimulation (one DC addition) and a 7-10 d co-incubation period[37,38]. Our data therefore strongly suggest that AAVs may be effective vectors for manipulating DCs[20,36,39].

DISCUSSION

Experimental algorithm for solid liver tumors

The key to the new evolution toward immunotherapy is to set up an algorithm for patients who will not respond to surgery. As shown in Figure 1, the liver tumor of selected patients is staged and the patients are directed to follow the clinical[8] or immunological algorithm. The experimental option is designed by taking specimens, with the tumor being preferably harvested laparoscopically or by means of the open technique. The next step is to insert modified antigens with carriers into the neoplastic cells. At this point, the choice is whether to inject them with DCs after leukopheresis, or by means of a virus (AAV). Both injections can be performed laparoscopically, thus allowing minimally invasive surgery, the introduction of the antigen inside the area of the tumor, and the initiation of a cell-mediated reaction designed to ensure immunological cytoreduction or debulking. An ultrasound-guided needle is placed into the abdomen, and a biopsy of the tumor can be taken.

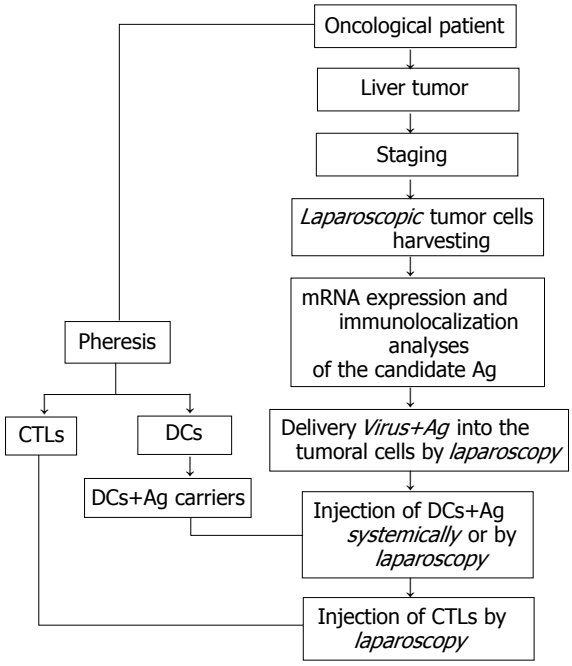

Figure 1 Relationships between laparoscopic surgery and immunotherapy for treatment of primary and secondary liver tumors. CTLs: cytotoxic T lymphocytes; DCs: dendritic cells; Ag: antigen.

As laparoscopy is a widely used surgical technique, most surgeons can easily adopt the procedure of injecting antigens, CTLs and DCs directly into the primary or secondary tumor in order to increase tumor immunogenicity and kill the remaining tumor cells by means of a local injection of CTLs and DCs for better cytoreduction. A port should be placed inside the internal jugular or subclavian artery in order to allow the retrieval of blood for leukopheresis; this port can be accessed quite easily using an external needle.

Improving cytoreduction: our new approach

The most widely used treatment is tumor resection, and so the main role of surgeons and oncologists is to decrease tumor bulk or mass in order to improve survival or allow the possibility of chemotherapy. Some liver tumors are so large that they are either inoperable or require such extensive surgery as to increase the incidence of death, but the introduction of cryoablation, alcohol injection, and radiofrequency ablation means that surgeons can reduce the amount of tumor in the liver, thus allowing chemotherapy to work on fewer tumor cells. The theory is that shrinking the tumor should lead to better chemotherapeutic results. Chemotherapy usually not only kills the tumoral cells remaining after radiofrequency, but also eliminates the satellite cells present in the liver or liver vessels together with good and normally replicating cells. We strongly believe that immunological therapy could become the new standard treatment for liver tumors. It not only debulks the tumor mass while destroying the tumor by means of a cell-mediated response, but its cell-

specific nature should enable it to kill satellite lesions and micro-metastases more efficiently (thus leading to better cytoreduction) without killing normal cells, and allow the use of low-dose chemotherapy to avoid or reduce undesirable side effects. Furthermore, the possible activation of memory responses could lead to much-needed long-term tumor immunosurveillance, which should reduce the incidence of relapses.

CONCLUSIONS

Evidence of practical immunotherapy treatment

Our initial clinical results suggest that there is an urgent need to explore further therapeutic options for liver tumors. This study introduces several innovations and a methodology that will help establish critical clinical assays for assessing immune responses targeting liver tissue, and verify the relationship between host response and liver tumor regression/progression.

Relevance to liver cancer research

We believe that immunological therapy can improve our overall understanding of how the host immune system interacts with primary and secondary liver cancer tissue, and will further elucidate useful methods for assessing this potential interaction.

Relevance to tumor immunology/immunotherapy

The importance of breaking host tolerance in order to achieve a tissue-specific mediated response and facilitate a favorable response to antitumor immunotherapy has recently been stressed in the literature[40]. For this reason, we believe that the use of a mini-invasive surgical approach, the immunotherapy and a clinical treatment will have a significant impact on liver tumors.

Costs and applications

Immunological therapy seems a very promising treatment for liver tumors. However, its specificity and cost makes it indicated for patients with tumors that cannot be resected by any of the different ablation methods, and small tumors in cirrhotic patients who are unsuitable candidates for standard surgery.

Improved cytoreduction

We believe that immunological therapy could become a new standard treatment of liver tumor for mainly three reasons: (1) it can debulk the tumor mass, while destroying the tumor by means of a cellular response; (2) it should be able to control the satellite lesions and micro-metastases more efficiently because of its more cell-specific nature, thus improving cytoreduction, avoiding the killing of normal cells, and reducing the use of chemotherapy and therefore its side effects; and (3) the possible activation of memory responses could lead to much-needed long-term tumor immunosurveillance, and thus reduce the incidence of relapses.

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Science Editor Guo SY **Language Editor** Elsevier HK