p53 gene in treatment of hepatic carcinoma: *Status quo*

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Abstract

Hepatocellular carcinoma (HCC) is one of the 10 most common cancers worldwide. There is no ideal treatment for HCC yet and many researchers are trying to improve the effects of treatment by changing therapeutic strategies. As the majority of human cancers seem to exhibit either abnormal p53 gene or disrupted p53 gene activation pathways, intervention to restore wild-type p53 (wt-p53) activities is an attractive anti-cancer therapy including HCC. Abnormalities of p53 are also considered a predisposition factor for hepatocarcinogenesis. p53 is frequently mutated in HCC. Most HCCs have defects in the p53-mediated apoptotic pathway although they carry wt-p53. High expression of p53 *in vivo* may exert therapeutic effects on HCC in two aspects: (1) High expression of exogenous p53 protein induces apoptosis of tumor cells by inhibiting proliferation of cells through several biologic pathways and (2) Exogenous p53 renders HCC more sensitive to some chemotherapeutic agents. Several approaches have been designed for the treatment of HCC *via* the p53 pathway by restoring the tumor suppression function from inactivation, rescuing the mutated p53 gene from instability, or delivering therapeutic exogenous p53. Products with p53 status as the target have been studied extensively *in vitro* and *in vivo*. This review elaborates some therapeutic mechanisms and advances in using recombinant human adenovirus p53 and oncolytic virus products for the treatment of HCC.

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Key words: p53 gene; Hepatocellular carcinoma; Therapeutic strategies; Advances; Prospects

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INTRODUCTION

The concept of human gene therapy^[1,2] derives from fundamental discoveries^[3,4] on the nature and working^[5] of the gene. Since the essential principles of molecular genetics and gene transfer in bacteria were established in the 1960s, gene transfer into animals and humans using either viral vector and/or genetically modified cultured cells has become inevitable^[6]. Since then, this concept has promoted thousands of researchers to attempt to realize their long-cherished dreams of eradicating some of the obstinate human diseases. Broadly defined, the concept of gene therapy involves transfer of genetic materials^[6] into cells, tissues, or whole organs, with the goal of eliminating diseases or at least improving the clinical condition of patients^[7]. As a milestone of gene therapy, the first approved clinical protocol started trials in September, 1990^[8]. Gene therapy provides great opportunities for treating diseases due to genetic disorders, infections and cancer^[9]. Hepatocellular carcinoma (HCC) is one of the 10 most common cancers worldwide with its highest prevalence in Southeast Asia and sub-Saharan Africa^[10]. Although great efforts have been made to overcome this cancer, no ideal treatment is available at present^[11]. In recent years, many researchers are trying to improve the effects of treatment by changing therapeutic strategies^[12]. As the majority of human cancers seem to exhibit either abnormal p53 gene^[13] or disrupted p53 gene activation pathways^[14], intervention to restore wild-type p53 (wt-p53) activities is an attractive anti-cancer therapy^[14,15]. This review elaborates some therapeutic mechanisms and advances in using recombinant human adenovirus p53^[16] and oncolytic virus^[17] products for the treatment of HCC.

CARCINOGENESIS OF LIVER CELLS AND p53 GENE

Cancer predisposition, onset and therapeutic response can be critically determined by the integrity of tumor suppressor p53^[15]. Tumor suppressor protein p53 can protect cells from growth and division^[18], thus mediating cell-cycle arrest^[19], DNA repair^[20] and apoptosis^[13,14,19,21] after its activation by multiple forms of cellular stresses^[19]. p53 mutations^[22] in plasma DNA are associated with several cancers, and abnormalities of p53 are also considered a predisposition factor for HCC (Table 1). Mutant p53 (mt-p53) may be a marker of HCC carcinogen exposure^[22]. For example, in aflatoxin B1-exposed patients^[23], R249S tumor protein p53^[24], one of the cancer-associated mutants, encodes p53, and is considered a

Table 1 Abnormal p53 status detected in HCC with probable mechanisms

Endogenous status of p53 or its pathways	Probable mechanisms
Mutation of p53 gene	Point mutation, allele deletion, insertion, etc.
Loss of or decrease in wt-p53 expression	Enhanced p53 degradation, complex formation
Hyperactivities of negative regulators of p53	No p53 mutation, over-expressed negative regulators of p53 attenuate its function
Increased diversity of p53 aberration	Progression of HCC
Presence of serum anti-p53 antibodies	Over-expression of wt-p53, presence of mt-p53, or both

Table 2 Approaches for restoring tumor suppression function of p53 in the treatment of HCC

Strategies	Therapeutic effects
Chemotherapy or radiotherapy	Inducing p53-dependent apoptosis in tumor cells with wt-p53
Supplying exogenous wt-p53 by gene delivery	Suppressing growth of both mutant and wild-type p53-containing tumor cells
Overexpression of ARF	Blocking p53 degradation pathways to induce p53 triggered tumor cell death
Interruption of MDM2-p53 interaction	Preventing MDM2-mediated p53 ubiquitination and degradation to restore transactivation
Molecules stabilizing the active conformation of the protein	Rescuing mt-p53 to restore p53 function

genetic alteration during hepatocarcinogenesis^[23]. Here R249S as a mutant of p53 core domain, is a structural mutation^[25], and constitutes one of the “hot spots” associated with cancer.

There is evidence that the level of p53 alterations is high in HCC, since it was reported that p53 increases the frequency of HCC prediction from 79.5% to 86.3%^[26], showing that serum concentration of p53 protein may be a convenient and useful non-invasive screening test for prediction of HCC. A study^[27] showed that attenuated p53 function and telomere-induced chromosomal instability play a critical and cooperative role in the progression of chronic liver damage to hepatocellular carcinoma. Loss of p53 expression or presence of abnormal forms of the protein is frequently associated with HCC cell lines. Bressac *et al*^[28] studied the p53 gene at the DNA, RNA, and protein level in seven human HCC-derived cell lines, and found that six of them show p53 abnormalities, suggesting that alterations in p53 may be important events in the transformation of hepatocytes^[29] into the malignant phenotype. p53 gene is frequently mutated in high-grade^[30] HCC. Inactivation of this multiple tumor suppressor gene plays an important role in the progression of chronic liver damage to hepatocellular carcinoma by directly or indirectly inducing chromosome instability, cell proliferation and neovascularization^[31]. Kondo *et al*^[32] performed dual-color fluorescence *in situ* hybridization to evaluate loss of the p53 gene, and revealed that loss of the p53 gene occurs in HCC, and diversity of the p53 gene aberration increases with the progression of chronic liver damage to HCC.

Other factors for hepatocarcinogenesis are correlated with abnormal functioning of p53, such as hepatitis B virus (HBV)^[23,33] and hepatitis C virus (HCV)^[34]. HBV gene encodes HBV protein x (HBx)^[33], a protein as a transcriptional activator^[35] and plays an important role in viral replication in HBV-infected cells. HBx as an oncoprotein^[36], can bind to the C terminus of p53 and inhibit several critical p53-mediated cellular processes, including DNA sequence-specific binding, transcriptional transactivation, and apoptosis^[37]. HBx integration and inactivation of p53 by mutations and regional allelic deletions are frequently found in tumors associated with HBV infection^[38]. HBx up-regulates survivin^[39] expression in hepatoma tissues. Survivin is an inhibitor of apoptosis and found in many common human cancers but not in

normal tissues. Survivin is suppressed by wt-p53 and over-expressed in 41%-70% of HCCs from Asia, its over-expression is associated with aberrant p53 nuclear positivity^[40].

In addition to its aberration, as p53 responds to a variety of genotoxic^[34] and cytotoxic^[41] agents in the presence of a potent inhibitor^[42] of p53, the liver's ability to handle such toxic agents is influenced, thus inducing hepatocarcinogenesis.

Anti-p53 antibodies (p53-Abs) are products triggered by accumulation of a mutated form of p53 protein and probably a large quantity of wt-p53 protein^[43]. As a specific serological marker for p53 gene expression changes in HCC, the presence of p53-Abs is independent of serum alpha fetoprotein and other conventional tumor markers^[44]. It was reported that serum p53-Abs have a specificity of 100% for detecting malignancy^[44], suggesting that its use in combination with markers may increase the diagnostic sensitivity of cancer.

MECHANISMS OF p53 THERAPY FOR HCC

Restoration of tumor suppression function of p53 has been speculated in several clinical lines for the treatment of HCC (Table 2).

p53 is frequently mutated in HCC^[31]. Most HCCs have defects in the p53-mediated^[37] apoptotic pathway although they carry wt-p53. Sometimes, p53 in HCC is wild-type but has inactive function^[45]. Signal to and activation of p53 can lead to wt-p53 expression, thus suppressing the transformed phenotype of hepatocytes and increasing the effects of both chemotherapeutic agents and radiation therapy^[46]. Repression of p53 can be partial or complete^[45] in HCC, and activation of p53 can be achieved by single small molecules, such as the well known antimalaria drug quinacrine^[45]. Supplying exogenous wt-p53 in cancer cells by gene delivery is effective in suppressing tumor growth of both mutant and wild-type p53-containing tumors^[14]. The murine double minute 2 gene (MDM2) is an oncogene and contains a p53-DNA binding site and produces a phosphoprotein that forms a tight complex with both mutant and wild-type p53 protein, thus inhibiting p53-mediated transactivation and inducing p53 degradation^[47]. There is a MDM2-p53 auto-regulatory feedback loop^[48] that regulates the function of p53 protein and expression

of the MDM2 gene. Expression of the MDM2 gene can be regulated by the level of wild-type p53 protein, while the MDM2 protein, in turn, can form a complex with p53 and decrease its ability to act as a positive transcription factor at the MDM2 gene-responsive element. Several approaches have now been used to interrupt MDM2-p53 interaction to increase functional p53 levels and p53-mediated therapeutic effectiveness^[49]. ADP-ribosylation factor (ARF) proteins are critical regulators of the protein secreting pathway^[50]. In a human HCC cell line^[51], adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are degraded to adenosine and treatment of HCC cells with adenosine can inhibit growth of HCC cells and activate caspase-3, indicating that adenosine is an apoptotic agent with cytotoxicity. ARF is considered a tumor suppressor^[52], and can initiate cellular response to aberrant oncogene activation by binding to and inhibiting the activity of MDM2. The human counterpart^[53] of MDM2 (HDM2), like the murine protein, can inactivate the transactivation ability of human p53. ARF and p53 bind to MDM2 on different sites. ARF-p53 complex is formed depending on the mediation by MDM2 and can enhance p53 stability^[54]. Blockage of p53 degradation pathways either by over-expression of ARF or interruption of MDM2-p53 interaction can effectively induce p53-triggered tumor cell death^[14].

High expression of p53^[55] *in vivo* may exert therapeutic effects on HCC in two aspects: (1) High expression of exogenous p53 protein induces apoptosis of tumor cells by inhibiting proliferation of cells through several biologic pathways and (2) exogenous p53 renders HCC more sensitive to some chemotherapeutic agents.

In addition, p53 down-regulates the expression of genes involved in angiogenesis^[56], by the angiogenesis-inhibiting properties of wt-p53 protein^[13].

It is very difficult to treat HCC due to drug resistance. It is necessary to reverse multiple drug resistance to human HCC cells and to find out the related mechanisms. The expression and activity of P-glycoprotein as well as the multiple drug resistance gene (MDR) products, are elevated in HCC cells with mt-p53^[57]. Expression of mt-p53 enhances drug resistance to HCC cells and reduces their uptake of chemotherapeutic agents. In contrast to the increased expression of MDR by mt-p53, wt-p53 inhibits transcription of MDR^[58]. Therefore, restoration of wt-p53 activity in HCC cells leads to the sensitivity of HCC cells to chemotherapeutic agents because of the decreased expression of P-glycoprotein encoded by MDR.

APPROACHES OF p53 THERAPY FOR HCC

Studies indicate that it is necessary to deliver therapeutic genes into cells with high specificity and efficiency in order to increase the effect of gene therapy against cancer^[5,7,9,11]. A key factor for the success of gene therapy is the development of delivery systems^[5,7,9] that are capable of efficiently transferring genes into a variety of tissues, without any associated pathogenic effects^[7]. These techniques permit the isolation and insertion of genes into the recombinant delivery systems^[5]. Two kinds of gene

transfer vectors, namely viral and non-viral, are available at present.

Viruses are recognized natural gene carriers^[5] and provide inspiration of gene therapy. Viruses have been engineered as gene delivery vectors^[59]. However, their limits such as selective disadvantage^[60], immunogenicity and toxicity^[61], inefficient gene transfer and short-lived^[62] or inadequate expression in transfected liver cells^[63], require us to search other delivery systems.

Non-viral vectors have several advantages over viral vectors^[61]. The transferred gene is in the form of a plasmid^[64] that is on the surface or in the interior of the vector. Such vectors include liposome^[61], molecular conjugates^[65], nanoparticles^[66] with strong anti-tumor effect on human HCC, naked DNA^[67] and complexed DNA^[68]. Better transfection efficiency can be achieved with delivery systems such as cationic lipids and cationic polymers^[63].

To date, tissue-specific expression^[69], self-replicating^[70] and integrating plasmid^[71] systems have been reported for gene therapy of HCC. Iodized oil emulsion^[72] has a particular affinity to hypervascular hepatic tumors and is now commonly used in HCC chemoembolization, suggesting that it can be applied to the liver as a non-viral gene transfer system for intra-arterial gene delivery with selective gene expression in tumor cells.

THERAPEUTIC PRODUCTS WITH p53 STATUS AS TARGET

Recombinant adenovirus p53 (rAd-p53) and oncolytic virus are the promising therapeutic products for the treatment of HCC mostly applied *in vitro* and *in vivo* at present. With the introduction of exogenous wt-p53 expressed by the recombinant adenoviral vector, the expression of both p53 and p21 proteins is found to be up-regulated in cells^[55]. Inhibition of cell growth and apoptosis can be achieved^[55]. Oncolytic viruses^[73,74] are a number of defective viruses, which cannot replicate in normal cells but are able to grow in tumor cells, finally leading to their lysis.

The two kinds of viruses and their characteristics were compared (Table 3).

Recombinant adenovirus p53 (rAd-p53)

Since human adenovirus vector systems have a larger host range and lower pathogenicity to humans^[75], and the binding affinity for epithelium^[76] is important because most of human tumors are of the epithelial origin, and serotype 4 of species E^[77,78] shows a specific binding affinity for HCC cells^[76], they are generally used for the expression of proteins in human beings or other species with some advantages. The E1 region of adenovirus has been identified as a subregion of the viral genome present in transformed cells and is responsible for transformation^[79]. Recombinant human adenoviruses constructed with the E1 region replaced by exogenous DNA become replication-defective and yield a relatively low degree of acute toxicity^[80]. Since recombinant adenoviral vector expresses wt-p53 (Ad-p53), p53 gene can be transfected into HCC cell lines^[55]. Experiments showed that tumor cells transduced with the wt-p53 gene can inhibit *in vivo*

Table 3 Comparison of several characteristics of rAd-p53 with oncolytic virus products

Products	Characteristics
rAd-p53	p53 gene is transfected into HCC cells with recombinant adenoviral vector expressing wt-p53
Advexin	Larger host range, low pathogenicity to human, replication-impaired adenoviral vector carrying the p53 gene
Gendicine	The first commercial gene therapy product in the world approved by SFDA
SCH58500	Replication-deficient type 5 adenovirus vector expressing human wt-p53 under control of cytomegalovirus promoter
Oncolytic viruses	Incapable of replicating in normal cells but selectively replicating in p53-defective tumor cells to lyse them
ONYX-015	Tumor-selective replicating virus, the prototype for oncolytic adenoviral therapy
CNHK300-mE	Replication-competent with advantages of both gene and virus therapies

tumor growth of adjacent nontransduced cells, suggesting that Ad-p53 is also anti-angiogenic^[81], partially by the bystander effect induced by the wt-p53 gene transfer on adjacent tumor cells.

Oncolytic viruses

As HCC cells with p53 defects have lost their cellular surveillance mechanisms, oncolytic viruses interfering with the main surveillance pathways such as those controlled by p53^[73], could replicate selectively in them and cause lysis. E1A gene of adenovirus is an apoptosis-inducing gene and E1B gene of adenovirus is an apoptosis-inhibiting gene. The 55-kilodalton (55kDa) protein from the E1B-region of adenovirus binds to and inactivates the p53 gene^[82]. Because of a deletion in E1B, the 55-kDa E1B protein is not expressed and the mutant adenovirus, termed ONYX-015^[83], is able to replicate only in wt-p53 deficient cells. The E1B55K-defective adenovirus ONYX-015 is a prototype^[73,82,83] of oncolytic viruses and can selectively replicate in and kill p53-deficient HCC cells, the success of cancer gene therapy is not promising unless it is carefully designed based on the biology of a specific^[84] tumor type. To enhance the efficiency of such oncolytic viruses, another E1B 55kDa-deficient adenovirus armed with a mouse endostatin gene has been constructed for anti-tumor activities against HCC, and termed as CNHK200-mE^[85]. With the synergistic effect of carrier virus and therapeutic gene, a novel approach has been established with the vector system termed as gene-viral vector^[86] or gene-viral therapeutic system wherein an anti-tumor gene is inserted into the genome of a replicative virus specific for tumor cells to combine the advantages of gene and virus therapies. Using the human telomerase reverse transcriptase (hTERT) promoter to drive the expression of adenovirus E1A gene and clone the therapeutic gene mouse endostatin into the adenovirus genome, CNHK300-murine endostatin (CNHK300-mE)^[87] is constructed, showing potential effects in the treatment of HCC.

EMPIRICAL STUDIES OF p53 THERAPY FOR HCC

Activation of p53 by either chemotherapy or radiotherapy induces p53-dependent apoptosis in tumor cells with wt-p53^[14].

In the treatment of HCC, p53 products are injected into liver tissue by a variety of routes^[55,72,82,88-92]. When p53 products are injected intratumorally^[55,88,89], introduction of exogenous wt-p53 can inhibit cell growth, the expression

of both p53 and p21 proteins is up-regulated in tumor cells. Intraarterial gene delivery into animal hepatic tumors can lead to selective gene expression in tumor cells^[72]. Antegrade intraportal and retrograde intrabiliary routes are compared, induce transgene expression in periportal areas of liver with no significant difference in transduction efficacy, and no apparent complications are observed apart from very mild elevation of serum biochemical parameters^[90]. The effect of bile and pancreatic juice on gene delivery has been studied under the guide of endoscopic retrograde cholangiopancreatography (ERCP)^[92], showing that neither bile nor pancreatic juice affects transgene expression. Intrasplenic injection of p53 products can transfer gene into the portal venous circulation. When p53 products are injected intravenously, barriers such as the endothelial lining of tumor vasculature impair the efficiency of adenoviral vectors for gene delivery into HCCs^[65,82], which can be overcome by direct injection of p53 products into tumor tissues^[89]. Other gene delivery routes have also been reported^[91].

High-volume hydrodynamic injection of a gene *via* the hepatic artery with inferior vena cava/portal vein occlusion can achieve a high level of gene expression in HCC rat model^[67]. This gene transfer technique may have potential in clinical gene therapy for HCC. Oncolytic adenovirus-mediated gene therapy induces tumor-cell apoptosis and reduces tumor angiogenesis, leading to inhibition of HCC growth in animal model^[88]. Because CNHK200-mE is capable of selectively replicating in HCC cells, thus suppressing tumor growth and antiangiogenic activity in nude mice^[85], it can be used as a potential agent in the treatment of HCC.

CLINICAL RESEARCHES OF p53 THERAPY FOR HCC

After *in vitro* and *in vivo* experiments, different adenovirus-mediated p53 gene therapies for various tumors have been evaluated^[46]. It was reported that when recombinant adenovirus p53 (SCH 58500) is administered by hepatic arterial infusion, and it distributes more predominantly in liver tissues than in tumors^[93].

Intratumoural injection of Ad-p53 can lead to over-expression of p53 in cancer cells by inducing cell growth arrest and apoptosis, and overcome resistance or increase the effectiveness of radiation therapy and chemotherapy^[94].

Hepatic artery embolization for the treatment of HCC was first reported in 1979^[95]. Since the 1980s, transcatheter arterial chemo-embolization (TACE) has been applied

to various HCCs apart from those with humoral hypercalcemia^[96]. TACE as a local ablative treatment is able to control local disease and prolong a similar survival to that of surgical resection^[11]. However, the recurrence of HCC after successful control of local tumor spread is high due to the non-curative procedure. In contrast to necrosis resulting from TACE, apoptosis is not commonly accompanied with inflammation that causes collateral cell damage, suggesting that the effects of intra-tumoral or intraarterial injection of p53 products in combination with TACE, on tumor tissue ischemia and necrosis, may be synergic and can improve survival.

In addition, inhibitors of p53-mediated apoptosis might be used to transiently decrease apoptosis in normal tissues when patients receive high doses of radiation or chemotherapy^[97]. Emulsion of iodized oil and contrast medium can be used as a nonviral gene transfer system for intraarterial gene delivery^[72].

CHALLENGES AND PROSPECTS

p53 is an ideal target for anti-cancer drug design^[14]. Blockage of p53 degradation pathways can effectively induce p53 triggered tumor cell death^[14]. Since unlike most other tumor suppressor genes, mt-p53 is over-expressed in tumor cells, a promising approach involving reactivation of tumor-suppressing function to mt-p53^[21,25]. Further understanding of the mechanisms of how to restore p53 activity, may lead to discovery of more potent analogs and new strategies^[11,73,91] for p53-targeting in tumor therapy.

More genes previously unknown have been identified that are involved in the regulation of p53 transcriptional activity and their over-expression inhibits p53 target promoters and p53-mediated apoptosis, suggesting that these genes play a role as p53 inhibitors and may have oncogenic activity^[98].

As hepatocarcinogenesis is a multistage process involving a number of genes^[99,100], attention should be paid to the target genes whose altered expression actually mediates neoplastic phenotype. There is an urgent need to establish simple and low-cost tests for detecting expression of p53-related genes in HCC^[99,100] that are hallmarks of HCC development. Continuous monitoring of serum p53 protein is important in early detection of recurrence of HCC, and immunodetection of serum p53 is valuable^[101] for post-operative monitoring during follow-up in preoperatively positive patients.

Nevertheless, about 40% of cancers retain wt-p53, and there may be mutations of other genes in cells with p53 mutations. Therefore, the mechanisms of p53 in HCC therapies should be further studied.

It was reported that hepatic arterial administration of p53 products cannot substantially increase transduction of tumor cells, and ligation of the hepatic artery following infusion of adenovirus or addition of lipiodol infusion has no effect on the transduction of tumor cells^[102], suggesting that better administration approaches must be developed for more efficient transduction of tumor cells.

Safety and research ethics must be emphasized. Human gene therapy can lead to serious adverse effects and even death^[103]. In a gene therapy experiment in 1999^[104], death

of Jesse Gelsinger was found to be directly linked to the viral vector used for the treatment. This tragic event has raised new questions about the prospects for human gene therapy, which not only achieves a therapeutic effect but also has potential adverse effects.

Another task at present is to find out the key points of tumor resistance to rAd-p53^[105-108]. Since exogenous wt-p53 was introduced, inhibition of tumor growth has become unnecessary for human cancer cells carrying mt-p53^[105]. In some cancer cells, wt-p53 is inactivated by different mechanisms. Since the presence of mt-p53 may induce genome instability of human cancer cells and mutator ability, they can escape the effects of exogenous wt-p53 and contribute to the failure of wt-p53 gene therapy. On the contrary, exogenous wt-p53 in other cancers can have effects only on those expressing wt-p53. For example, alterations of p53 gene are uncommon in differentiated thyroid neoplasm but can be frequently detected in anaplastic thyroid carcinoma^[107], suggesting that impaired p53 function may contribute to the undifferentiated and highly aggressive phenotype of these tumors. It was reported that exogenous expression of wt-p53 has influence on thyroid tumorigenic properties only in cells bearing altered p53, whereas it has no effect on cells expressing wt-p53 activity^[106], indicating that the endogenous p53 status seems to be essential for the effectiveness of p53-based gene therapy for some cancers. Another study^[108] evaluated the therapeutic effects of truncated Bid gene (tBid) on p53-resistant HCC and demonstrated that this gene only targets AFP-producing cells but not non-AFP producing ones^[109]. The introduction of tBid can not only significantly but also specifically kill HCC cells that produce AFP, indicating that death of HCC cells is induced by an apoptotic pathway independent of p53 status^[108].

CONCLUSION

p53 is an ideal target for the design of anti-cancer therapeutic strategies. p53 is frequently mutated in a significant portion of HCCs, and can mediate defective apoptotic pathways in HCC carrying wild type of p53. Several approaches have been designed for the treatment of HCC *via* the p53 pathway by restoring tumor suppression function from inactivation, rescuing mutated p53 gene from instability, or delivering therapeutic exogenous p53. Products with p53 status as the target have been studied extensively *in vitro* and *in vivo*. Although their therapeutic effects are limited, further study is needed to elucidate the mechanisms of p53 in HCC therapies, the role of endogenous p53 status, novel genes involved in the regulation of p53 transcriptional activity, the establishment of simple and low-cost tests for detecting expression of p53-related genes in HCC, better administration approaches of exogenous p53, safety and research ethics, and the key points of resistance to rAd-p53 in HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is one of the 10 most common cancers

worldwide. There are no ideal therapies for advanced HCC so far, and many researchers are trying to improve the effects of treatment by changing therapeutic strategies including application of gene therapy.

Research frontiers

About 50% of human cancers are associated with mutations in the core domain of tumor suppressor p53, thus p53 is regarded as the most frequently mutated gene. It is an attractive approach to the treatment of HCC by restoring wild type p53 activity.

Innovations and breakthroughs

Several approaches have been designed for the treatment of HCC via the p53 pathway by restoring tumor suppression function from inactivation, rescuing the mutated p53 gene from instability, or delivering therapeutic exogenous p53. Products with p53 status as the target have been studied extensively *in vitro* and *in vivo*.

Applications

p53 pathway can be used as a target in the treatment of HCC.

Terminology

Gene Therapy: The treatment of certain disorders, especially those caused by genetic anomalies or deficiencies, by introducing specifically engineered genes into patient cells. **p53 gene:** It is a tumor suppressor gene providing instructions for making a protein called tumor protein 53 (TP53). Through the effect of the protein it produces, TP53 is a tumor suppressor gene regulating the cycle of cell division by protecting cells from growing and dividing too fast or in an uncontrolled way. The p53 protein is located in the nuclei of cells throughout the body and can bind directly to DNA. When the DNA in cells becomes damaged, this protein plays a critical role in determining whether the DNA is repaired or the cells undergo programmed cell death (apoptosis). If the DNA can be repaired, p53 activates other genes to repair the damage. If the DNA cannot be repaired, the p53 tumor protein protects cells from dividing and signals it to undergo apoptosis. This process protects cells with mutated or damaged DNA from dividing, which helps prevent the development of tumors. Because the p53 tumor protein is essential for regulating cell division, it has been nicknamed the "guardian of the genome". **Vector:** Any device of transportation or movement. In this article, it denotes "a virus used to deliver genetic material into cells" or "a piece of DNA carrying DNA fragments into host cells".

Peer review

This review elaborates some therapeutic mechanisms and advances in using recombinant human adenovirus p53 and oncolytic virus products in the treatment of HCC. This review is helpful for many readers. The prospects should be expanded.

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