

Gene Therapy for Hepatocellular Carcinoma: An Update

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Abstract

Current statistics indicate that hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third leading cause of cancer-related death. Major predisposing conditions are hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. To date treatment approach includes liver transplantation, surgical resection and or ablation, however; recurrence, metastasis, and mortality still remains high. Therefore, alternative treatments such as gene therapy is increasingly being considered as a feasible proposal. In this mini review we will focus on novel data of the past 10 years on the subject of gene therapy and hepatocellular carcinoma.

Key words: vectors, hepatocellular carcinoma, gene therapy

Introduction

Multimodality treatment with surgery, radiotherapy and chemotherapy still remains the cornerstone of cancer treatment. Based on the stage during diagnosis neo-adjuvant or adjuvant treatment is applicable for several cancer types. Unfortunately chemotherapy and radiotherapy have adverse effects and in several situations the patients' treatment has to be postponed until these fade away. Most common adverse effects arise from bone marrow suppression [1]. In several cases bone marrow suppression requires hospitalization [2, 3]. Therefore novel routes of administration and treatments have been investigated for many types of cancer, with the main concept being the local treatment [4-14]. Chemotherapy is administered in most cases

intravenously and in some cases orally. However, breakthroughs in revealing the genome mutations of tumors have identified specific mutated populations where targeted therapy can be administered orally [15-17]. Multiple genetic mutations and pathways are currently being investigated for several cancer types of liver cancer [18]. In these populations certain pathways which are overexpressed have been targeted with novel drug formulations [19-21]. Pharmacogenetics is currently the tip of the arrow for novel drug, design, development and therapy [22]. Unfortunately there are several different genetic profiles with very few patients for each mutation. In this group of patients new mutations can occur after targeted treatment, but again new inhibitors can be

administered [23]. Unfortunately there are resistance mechanisms that tumors develop therefore new strategies are used to sensitize tumors to chemotherapy and radiotherapy [24]. Another less toxic therapy that is currently being investigated is gene therapy. Suicide gene therapy (a type of gene therapy) has the ability to convert a non-toxic drug which penetrates the tumor, to cytotoxic. The conversion of the pro-drug to active drug takes action inside the tumor with the help of an administered viral or bacterial gene. Most importantly the normal cells are not affected [25-27]. To date suicide gene therapy has been investigated in: a) liver [9, 28, 29], b) colon [8, 30, 31], c) neuroendocrine [32], d) lung [33, 34], e) medulloblastomas [35], f) spinal cord tumors [36], g) prostate [37], h) breast [38, 39], i) bladder [40], j) brain [41], k) head and neck [42], l) gliomas [43-45] and m) sarcomas [46]. It has been observed that suicide gene therapy is efficient in chemotherapy resistant cancer cell lines [47] and can enhance radiotherapy [48]. In a recent study it was observed that micrometastasis were efficiently controlled with suicide gene therapy [49]. Moreover; suicide gene therapy is being explored by using technology such as nanoparticles to efficiently penetrate any tumor microenvironment [50-52] (Figure 1). In the current mini review article we will focus on hepatocellular carcinoma and the current knowledge of the last 10 years.

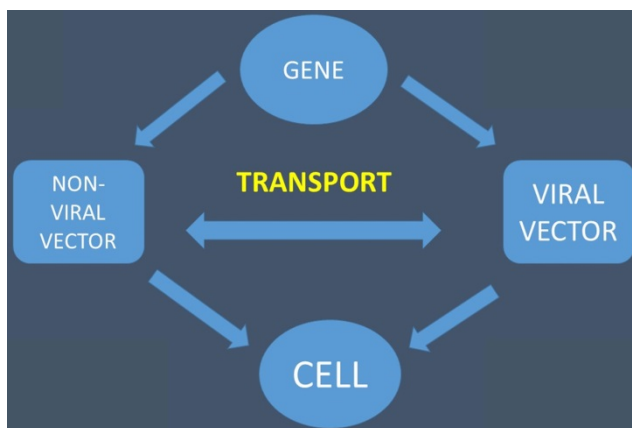


Figure 1. The gene is transported with the help of viral and non-viral vectors to the cell

Research Strategies

We performed an electronic article search through PubMed, Google Scholar, Medscape, and Scopus databases, using combinations of the following keywords: cancer gene therapy, bystander effect, suicide gene therapy, gene therapy, suicide gene therapies strategies, vectors for gene therapy. All types of articles (randomized controlled trials, clinical observational cohort studies, review articles, case

reports) were included. Selected references from identified articles were searched for further consideration, without language limitation. We focused mostly on data published in the past 10 years.

Hepatocellular

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and currently ranks third among the most prevalent deadly cancers in the world [53]. The incidence of HCC will rise in the next years due to the increasing prevalence of hepatitis C virus (HCV) in Europe, North America and Japan. Moreover; the risk for HCC development is now known to be age-dependent [54]. To date surgery and transplantation remains the only potentially curative modality, however; the recurrence rate is high and there is poor long-term survival [55]. Furthermore, radiotherapy (RT) is an additional treatment modality to treat hepatoma patients [56]. However, again RT treatment in HCC patients suffer from recurrence problems due to radio-resistance acquisition. Radio-resistance still remains a serious impediment to successful treatment of HCC patients [57].

Studies

In the study by Sia KC et al. [9] the hepatocellular cancer cell line was cultured to evaluate a suicide gene therapy model. The herpes simplex virus type 1 (HSV-1) amplicon viral vector was coupled with yCD 5-FC and was administered. It was observed that HCC 26-1004 tumor xenograf expressed high levels of y-CD genes with non-invasive imaging. The firefly luciferase reporter gene is a valuable tool to monitor the activity and bio distribution of the therapeutic gene expression, however; it cannot be used in clinical trials as it is not bio-compatible. In the same study it was proposed by the authors radiation to be used along with 5-FU as it known to be a radiosensitizer agent [58]. In the study by Lin L et al. [59] it was observed that γ -Glutamylcysteine synthetase (γ -GCSH) could represent a prime target for overcoming resistance of anticancer drugs and radiotherapy for HCC cells. In the study by Luo X et al. [60] it was observed that stress-induced phosphoprotein 1 (STIP1) is up-regulated in the HCC tissues. STIP1 plays an oncogenic role in the progression of HCC, and it was suggested that STIP1 might be a therapeutic target. In the study by Xu D et al. [61] the results indicated that the promoter hypermethylation of GNAO1 might play an important role in HCC. GNAO1 might also be used as a biomarker for hepatocellular diagnosis and a future targeted therapy. In the study by Ceballos MP et al. [62] Sirtuins (SIRT) 1 and 2 deacetylases were overexpressed in hepatocellular carcinoma and were

associated with tumoral progression and multidrug resistance. It was supported that downregulation of the expression of P-gp and MRP3 supports the potential application of SIRT1 and 2 inhibitions in combination with conventional chemotherapy. In the study by Zhuang H et al. [63] it was reported that Glycine decarboxylase (GLDC), an oxidoreductase, plays an important role in amino acid metabolism. The data of the study indicate that GLDC downregulation decreases ROS-mediated ubiquitination of cofilin and as a result enhances hepatocellular progression. In the study by Qiu Z. et al. [64] the findings of the study indicated that urolithin A exerted an antiproliferative effect by regulating the Lin28a/let-7a axis and may be a potential supplement for HBV-infected HCC therapy. In the study by Liu X et al. [65] the findings suggested that Stellerachamaejasme L (ESC) regressed growth and metastasis of human hepatocellular carcinoma, by downregulating microRNAs expression. In the study by Yang B. et al. [66] experiments and clinical samples demonstrated a dynamic network biomarker (DNB) with calmodulin-like protein 3 (CALML3) reduced pulmonary metastasis in liver cancer. The loss of calmodulin-like protein 3 predicted shorter overall survival and disease free survival in postoperative HCC patients. Therefore it could be used as a prognostic biomarker and a future therapy target in HCC. In the study by Sheng J et al. [67] it was reported that various chemotherapeutic drugs for HCC treatment can increase autophagic flux of HCC cells. Moreover; it was related with enhancing drug resistance and promoting cell survival. It is known that most hepatocellular patients are insensitive to chemotherapeutic drugs, and resistance usually develops after a few sessions of chemotherapy treatment. Autophagy induction is a frequent response of HCC cells to chemotherapeutic drugs and induces acquired resistance. In the study by Ye Y et al. [68] insulin-like growth factor receptor-1 (IGF1R) was identified as a direct target gene of miR-495 in hepatocellular carcinoma. Insulin-like growth factor receptor-1 was upregulated in hepatocellular tissues and negatively correlated with miR-495 expression level. The upregulation of insulin-like growth factor receptor-1 rescued the miR-495-induced tumor-suppressive roles in hepatocellular carcinoma cell proliferation and invasion. Finally, restored miR-495 expression and inactivated the protein kinase B and extracellular regulated protein kinase signaling pathway. These results indicate that miR-495 may be a novel therapeutic target for patients with hepatocellular carcinoma. In the study by He RQ et al. [69] the potential role of miR-23b-3p in hepatocellular carcinoma tumorigenesis and progression was

elucidated. Moreover; miR-23b-3p it was suggested that it may act as a predictor of HCC. In the study by Xia Y et al. [70] the HA-Se-PEI@siRNA was internalized into the HepG2 cell mainly in a clathrin-mediated endocytosis manner. The treatment with HA-Se-PEI@siRNA resulted in greater antitumor efficacy compared with the Se-PEI@siRNA in vitro and in vivo. Moreover; HA-Se-PEI@siRNA was almost no toxic to kidneys, lungs, heart and liver of mice. In the study by Wang L et al. [71] the results of the study indicated that alpha induced protein 8 like 2 (TIPE-2) acts as an inhibitor of HCC cell growth and promotes apoptosis. Alpha induced protein 8 like 2 may inhibit the metastasis-associated PI3K/AKT signaling cascade and may downregulate the tumor cell cycle. These findings provided the mechanism by which TIPE-2 promotes apoptosis of hepatocellular carcinoma. In the study by Liu J et al. [72] it was revealed that the growth of tumor was significantly suppressed after the transfection of T-cell immunoglobulin and mucin-domain containing-3. In the presence of T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), the proliferation of splenocytes and cytolysis in the early phase of tumor development was significantly enhanced. Moreover; the antitumor effect was further improved by the synergistic effect of Tim-3 with Transporter associated with Antigen Processing 1 (TAP1). Therefore, the membrane-type Tim-3 is an effective immunoregulator and enhances antitumor immune response. In the study by Bai G et al. [73] cyclin dependent kinase 1 (CDK1), NDC80, cyclin A2 (CCNA2) and rac GTPase activating protein 1 (RACGAP1) were shown to be targeted by the HCV nonstructural proteins NS5A, NS3 and NS5B, respectively. It was observed that the four genes perform an intermediary role between the HCV viral proteins and the dysfunctional module in the HCV key genes interaction network. In the study by Xue Y et al. [74] the results of the study suggested that the shRNA-mediated knockdown of Frizzled-7 (FZD7) induced apoptosis of HCC lines through the inhibition of nuclear factor- κ B (NF- κ B). In addition, the transforming growth factor (TGF- β)/Smad signaling pathway appeared to participate in the underlying mechanism of FZD7 in hepatocellular carcinoma cell lines. In the study by Li M et al. [75] results indicated that deguelin could inhibit hepatocellular carcinoma cell lines through suppression of angiogenesis and reduce proangiogenic factors in cancer cells. In the study by Dhanasekaran R et al. [76] global gene expression profiling revealed engagement of miR-17 target genes and inhibition of key transcriptional programs of family of regulator genes and proto-oncogenes that

code for transcription factors (three related human genes: *c-myc*, *l-myc*, and *n-myc*. *c-myc* called MYC). Therefore, anti-miR-17 is an effective therapy for MYC-driven hepatocellular carcinoma cell lines. In the study by Ogura S et al. [77] the data indicated that FoxM1 links the mevalonate pathway to oncogenic signals in hepatocellular carcinoma cell lines. The results indicated that a novel therapeutic approach to inhibit FoxM1 by targeting the mevalonate pathway for hepatocellular carcinoma cell lines is feasible.

Discussion

In order for gene therapy to work specific vectors or pro-drugs have to be chosen for each cancer type. Novel vectors and transporters of genes are in need. Currently the thymidine-active mutant of dCK, dCK.DM.S74E was created which activates multiple pro-drugs such as; BVdU, LdUNAs and LdT. It has been observed that the system has the ability to sensitize and re-sensitize tumors to chemotherapeutic agents. Moreover; it was observed that it can activate more than one pro-drug simultaneously and prevents multidrug resistance without any additional side effects [78]. Another method to enhance gene therapy is local application to the tumor site [6, 79]. There are cases where loco-regional administration is not possible and therefore systematic administration has to be chosen. We need drugs that have a sustain release effect. The concept relies on the "stealth" ability of several molecules to circulate within the blood stream without being detected and have a sustain release gene expression effect.[80, 81] In the study by Zhao Y et al. the neural stem cells (NSCs) derived from hairy and enhancer of split-1 (HES1) human embryonic stem cell line had the ability to migrate from the injection site. In order for the administration system to work a baculovirus vector was used to insert the HSV-tk suicide gene into the cells. Moreover; ganciclovir was co-administered in order for an amount of concentration to be present locally. Indeed the transgene expression was present for three weeks [43]. The same concept was also applied with mesenchymal stem cells (MSC) in a hepatocellular carcinoma cell lines model [82]. In the study by Wang C et al. [41] investigated neural stem cells (NSCs) (F3) as dual suicide gene therapy with Cytosine Deaminase (CD) and Thymidine Kinase (TK) creating the NSC-F3.CD-TK. Enhanced antitumor activity against lung cancer metastasis in comparison to single suicide gene therapy was observed. In another study lung cancer cell lines were used along with a carcinoembryonic antigen (CEA) promoter with Thymidine Kinase and Cytosine Deaminase constructing the pCEA-TK/CD [34]. Dual suicide gene therapy has been investigated with

surviving promoter Ad-survivin/GFP and Ad-survivin/CD/TK and as anticipated higher efficiency was observed compared to single suicide gene therapy [83]. Moreover; combination suicide gene therapy has been investigated with (VSV)-MΔ51-expressing (CD::UPRT)-5FC in four different cancer cell lines (Prostate PC3, Breast MCF7, TSA mammary, Adenocarcinoma, B-Lymphoma Karpas-422, and Melanoma B16-F10) and as a result increased tumor oncolysis was observed [84]. The combination treatment was more effective against the parental mammary adenocarcinoma (TSA). Unfortunately dual gene therapy is not applicable for all cancer types. In the study by Tang Q et al. [52] local treatment was enhanced in a human hepatic cancer cell line model by intratumoral administration of KDR-TK and *Alpha-fetoprotein* -*Thymidine Kinase-Luciferase* Knockin Mice (AFP-TK) with microbubble contrast agent prior to ultrasound treatment. The pro-drugs 5-Fluoracil (5-FC) and ganciclovir (GCV) were administered after the intratumoral therapy. Between the two groups there was no difference in the antitumor activity. The time of the pro-drug administration is crucial for the efficiency of the treatment as the pro-drug has to be already diffused within the target tissue for the administered gene therapy to be effective. In the study by Duan X et al. [50] gene transfection of the novel cationic self-assembled DOTAP and MPEG-PCL hybrid micelles (DMP) was investigated. The new transport had less toxicity compared to the polymer Polyethyleneimine (PEI) with 25kDa. In the present study DMP delivered efficiently the urvivin-T34 gene (S-T34A) to treat C-26 colon cancer cell lines (CLC). Nanoparticles have been used previously in other gene transfection studies [85, 86]. To date there are very few studies with suicide gene therapy in clinical trials and every effort towards [6, 87-99]. Recently a clinical trial for prostate cancer was published and others in extensive stage followed [37, 96]. In a recent study the suicide gene TK.007, was used and demonstrated efficiency in several cancer cell lines (G62 human glioblastoma cell line, SW620 human colorectal adenocarcinoma cell line, A549 human lung carcinoma, and IPC298 human melanoma cell line) [100]. It was observed that TK.007 had higher gene transfection in comparison to HSV-tk with lower doses of ganciclovir. The spliceosome-mediated RNA trans-splicing technology has the ability to replace a tumor-specific transcript with one encoding a cell death-inducing peptide/toxin. This technology enhances the gene transfection. In another study by Gruber C et al. [101] the efficiency of 3' *pre-trans-splicing molecules* (PTM) was investigated against highly malignant tumors. The group of Di

Stasi et al. [102] developed a new system that targets the inducible caspase 9 (iCasp9) gene. Firstly it was developed for children who developed graft-vs.-host disease (GVHD) by donor lymphocytes. The process was fortunately reversed with the novel therapy. Receptors on living cells have been used as targets for gene therapy, such as; vascular endothelial growth factor (VEGF) [51, 85] and carcino-embryonic antigen (CEA) [34]. Moreover; there are other receptors that can be targeted for HCC gene therapy such as; a) cluster of differentiation (CD44s) [103], b) epidermal growth factor receptor (EGFR) [104], c) folate receptor (FR) [105], d) stage specific embryonic antigen 4 [106], e) cluster of differentiation [107], f) transferrin receptor (TfR) or cluster differentiation 71 (CD71) [108], g) mucins [109], and h) tumor resistance antigen 1-60 (TRA-1-60) [110] (Table 1). In the future we want small molecules that can be used as carriers for gene therapy and have a sustain release effect either for local treatment or systemic treatment. Additional modalities such as; radiotherapy could be added prior to local treatment.

Table 1. Pathways and Promoters

Targeted Pathways
-Epidermal Growth Factor Receptors
-Vascular Endothelial Growth Factor
-Carcino-embryonic antigen
-Transferrin receptor
-Mucins
-Cluster differentiation 44
-Cluster differentiation 133
-Stage specific embryonic antigen 4
-Tumor resistance antigen 1-60
Promoters
-Epidermal Growth Factor Receptors
-Transferrin receptor
-Carcino-embryonic antigen
-Prostate specific antigen
-Telomerase-hTERT
-Cyclooxygenase
-Cytokeratin 18-19

Competing Interests

The authors have declared that no competing interest exists.

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