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Review

Delivery Approaches of Gene Therapy in Hepatocellular Carcinoma

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Abstract. *Gene therapy has the potential to provide therapeutic benefits to hepatocellular carcinoma (HCC) patients and has been the subject of intense pre-clinical and clinical research in recent years. In HCC, delivery of gene therapy has been attempted through multiple routes, using many vectors and genes in both animal models and patients. Unfortunately, a highly effective gene therapy for HCC has not been reported so far. The efficiency and selectivity of the gene transfer to the tumor tissue is too low. A great proportion of the failure can be attributed to the gene/vector complex itself. However, there is certainly a critical role played by the delivery technique. In the last decade a large amount of studies has been conducted to develop the ideal gene delivery technique for HCC, though questions regarding safety, repeatability, and efficiency still linger. The aim of this article is to review gene delivery techniques for HCC. It focuses on the relationship between the gene/vector complex and the delivery technique at promoting efficacy of gene therapy, without the cost of unacceptable systemic toxicity. The delivery techniques include systemic intravenous (IV) injection, intra-arterial (IA) injection, intra-tumoral (IT) injection, intra-portal (IP) injection, intra-biliary (IB) delivery and intra-splenic (IS) injection. The relative merits of each of these techniques are herein analyzed and discussed.*

HCC is the fifth most commonly diagnosed cancer worldwide, with increasing incidence and is the third most common cause of cancer death (1, 2). Although a variety of treatment options, including chemoembolization, percutaneous ablation, molecular-targeted therapy, radiotherapy, surgical resection and liver transplantation, are currently available in clinical practice, the prognosis for this cancer type is still poor. The rates of recurrence for HCC in patients with underlying cirrhosis are very high (3-5). Therefore, novel therapeutic strategies, such as gene therapy, are being developed.

Gene therapy for HCC is based on the transfer of genetic material to tumor cells, to induce a therapeutic effect. It could complement or substitute current treatment options for HCC. When gene therapy is performed, efficient gene transfer to the tumor cells and minimization of transfer to normal cells is essential (6). Especially in HCC patients, the ability to avoid expression in normal hepatocytes is particularly important owing to the impaired liver function typically seen in this patient population. As most HCC patients suffer from severe liver disease, additional impairment of liver function, due to direct toxic effects gene therapy, may pose a serious risk (7, 8).

For efficient gene transfer the integration of many fundamental factors are required. These include the appropriate choice of a therapeutic gene (*i.e.* tumor suppressor genes, suicide genes, immunomodulatory genes *etc.*) and the selection of a safe and effective cell entry strategy (*i.e.* viral vector or non-viral vector). Also, the optimal delivery technique remains fundamentally important (9).

In this review, all currently available and emerging delivery techniques were considered. Literature was searched through the Pubmed literature search tool. Only articles in English were included. The key words mainly focus on delivery technique.

Current Delivery Techniques

Several delivery techniques for gene therapy in HCC have been studied, both in pre-clinical and clinical experiments. These include systemic intravenous (IV) injection, intra-

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arterial (IA) injection, intra-tumoral (IT) injection, intra-portal (IP) injection, intra-biliary (IB) delivery and intra-splenic (IS) injection.

Intravenous injection. Technically, IV is the most straightforward way to perform gene therapy, however, IV gene delivery to treat HCC faces a variety of difficulties (10). They include (a) interaction with blood components, (b) filtration by the pulmonary vascular bed, (c) uptake by the reticuloendothelial system, and (d) the most notable problem: non-specificity of HCC targeting, which may cause low efficacy and high toxicity. The transfection complex has to be reasonably inert with regard to unspecific interactions with normal tissue cells, the extracellular matrix and biological fluids, but should be able to bind specifically to the hepatoma cells (11).

The commonest applied strategy to circumvent this potential problem is transcriptional-targeting of HCC. Tumor-specific promoters (TSPs) restrict the expression of therapeutic genes only to hepatomas. By doing so, expression of a therapeutic, but potentially toxic, transgene will be inhibited in normal cells, where these promoters are not active (12, 13). Numerous TSPs have been used for HCC, such as angiopoietin-like-3, cytochrome *P450*, apolipoproteinB and others (14).

Wolschek *et al*. injected polyethylenimine (PEI)-based DNA complexes into human HCC xenograft-bearing severe combined immunodeficiency mice (SCID mice). The complexes shielded by covalent attachment of polyethylene glycol (PEG), make use of epidermal growth factor (EGF) as a ligand for targeting gene delivery to EGF receptor-expressing human HCC cells. Following injection, luciferase expression was predominantly found in the tumor, with levels up to 100 times higher than in the liver, which was the highest expressing major organ. Histological investigation showed reporter gene expression (β-galactosidase) localized to tumor cells. In the liver, DNA was taken up almost exclusively by Kupffer cells and subsequently degraded.

However, the most specific and well-characterized TSP in HCC is alfa-fetoprotein (AFP). Numerous groups have exploited the AFP promoter for transcriptional targeting of therapeutic genes in HCC, such as truncated BH3 interacting death agonist (tBid), factor-related apoptosis-inducing ligand (TRAIL), interleukin-2 and herpes simplex thymidine kinase, by either viral vector or non-viral vector for targeted gene expression (15-20).

Li *et al*. developed a promising HCC-targeted gene therapy vector driven by liver cancer-specific a-fetoprotein promoter/enhancer. They integrated the AFP promoter with and without its enhancing elements into the VP16-GAL4-WPRE4 Integrated Systemic Amplifier (VISA) vector to express BikDD, a mutant that mimics the constitutively active pro-apoptotic Bik protein from the Bcl-2 family which has been shown to possess potent antitumor activity, and tested the newly-constructed vector for expression efficiency and cell-killing activity. The activity of this expression vector is comparable with or even higher than that of strong cytomegalovirus (CMV) promoter. It exhibits strong promoter activity in liver cancer cells/tumors, but has nearly no or very low activity neither in normal cells/organs *in vitro*, nor in orthotopic animal models *in vivo*. In addition, targeted expression of a therapeutic BikDD effectively and preferentially killed liver cancer cells, but not normal cells. It significantly repressed growth of HCC tumors, and prolonged survival in multiple xenograft and syngeneic orthotopic mouse models of HCC through intravenous systemic gene delivery. Importantly, systemic administration of BikDD exerted no acute toxicity compared with CMV-BikDD in mice.

Furthermore, to overcome the limitation of the relatively low levels of transcription induced by TSPs, some strategies have been applied to enhance the expression of genes (21). Ahn *et al*. developed a bi-directional, two-step transcriptional amplification (TSTA) system driven by the tumor-specific survivin promoter (pSurv) to amplify the correlated expression of both the reporter gene firefly luciferase (FL) and therapeutic gene TRAIL. In the first "activator" step of this system, the chosen weak promoter directly drives the expression of a fusion protein between the GAL4-DNA-binding domain and two tandem VP16 transactivation domains. In the subsequent "effector" step, the GAL4-VP16-2 fusion protein promotes strong expression of a therapeutic gene under the control of multiple GAL4-binding sites and a E4TATA minimal promoter. The authors compared the specificity and potency of an adenovirus carrying this system (Ad-pSurv-TSTA-TRAIL-FL) to a non-specific vector (Ad-cytomegalovirus promoter (pCMV)-FL) in an orthotopic HCC rat model after systemic administration. At 24 h after injection of Ad-pCMV-FL, bioluminescence imaging revealed a trend towards greater FL expression in liver *vs*. tumor. In striking contrast, Ad-pSurv-TSTA-TRAIL-FL showed an increased FL activity within the tumor compared with the liver ($p<0.01$), a trend towards reduced liver expression compared with Ad-pCMV-FL ($p=0.07$), and importantly, similar FL levels within tumor compared with Ad-pCMV-FL ($p=0.32$).

Although the studies on TSPs are promising with regard to highly specific expression of a transgene in HCC tumors and no acute systemical toxicity, there are still some challenges with this strategy, which may negatively impact on the application of the IV gene delivery technique. Up to 40% of all HCC's do not produce AFP, which is the most well-characterized HCC TSP (22-23). If HCC TSP is negative, the transduction will be low. Even when genes, exhibiting a strong bystander or immunomodulatory effect, which may compensate low transduction efficiency, are being used (24-26).

Therefore, unless a novel high-specific and high-expression gene/vector complex emerges, the utilization of the IV route still has a long way to go before making the leap from bench to bedside.

Intra-arterial injection. Unlike normal hepatocytes that receive dual blood supply from the hepatic artery and the portal vein, HCC receives its blood supply mainly from the hepatic artery (27). Based on such a specific feature of HCC, IA administration may be a better-targeted approach for gene therapy. Due to the small size of the vasculature, it is difficult to perform this technique in animal models. Nevertheless, several reports with promising results have been published. Lu *et al*. (28, 29) injected a transferrin-p53-lipofectamine complex into the hepatic artery of rabbits to analyze the therapeutic *p53* gene transfer efficiency *in vivo* by western blot and immunohistochemical/immuno-fluorescence staining analysis. The results showed that transferrin receptor expression in hepatomas was 3-times higher than in normal hepatocytes, without apparent toxicity. Besides animal experiments, some early clinical feasibility studies have been performed as well. Chen *et al*. (30) showed that sequential therapy of *p53* gene transcatheter arterial infusion was safe and effective in humans. In 30 HCC patients they infused *p53* gene (10^{12} virus particles) combined with hydroxycamptothecin (20 mg) in the hepatic artery, once a week, for 3 weeks. In the control arm (18 patients) they infused only hydroxycamptothecin (20 mg). After 1 to 8 courses, the mean survival time (199.6±111.8 days and 83.0±50.2 days, respectively), and changes in the tumor size were significantly different between the 2 groups ($p<0.05$).

In contrast, Prieto's group indicated some limitations in the IA approach (31). They administrated adenovirus-expressing lacZ (AdCMVlacZ, $2*10^{10}$ plaque-forming unit per rat) IA and IT respectively in rats with diethylnitrosamine (DENA)-induced HCC. Liver specimens were obtained and stained with H&E (hematoxylin-eosinstaining) and X-gal. Then gene transfer efficiency was evaluated in neoplastic nodules of different size. The study showed that the IA route allowed efficient transduction of nodules up to 2-5 mm in diameter. Tumors greater than this size were resistant to transduction by an IA route, but could be transduced by direct intra-tumoral injection. The authors suggested that a physical barrier between the blood compartment and the tumor cells may exist, that could be responsible for poor transduction of tumor nodules, using an intra-vascular approach. This could be an important obstacle for IA application of gene therapy in HCC.

Another limitation of IA approach is the blood flow in the hepatic artery. In hypervascular HCC it may be too fast to allow efficient gene transfer. More recently, a novel technique was introduced to solve this dilemma: IA gene delivery in combination with hepatic arterial embolization. Kim *et al*. prepared four nonviral gene transfer systems by using pCMV-luc+ as a reporter gene (10). The first system consisted of a DNA and polyethylenimine (PEI) complex; the second, of a DNA and PEI complex mixed with iopamidol and iodized oil; the third, of a DNA and PEI complex mixed with iopamidol; and the fourth, of a DNA and PEI complex mixed with iodized oil. They delivered these cocktails IA to rabbits, 20 days after VX2 tumor implantation. Luciferase activity in the tumor was significantly higher for the group that received DNA, PEI, iopamidol and iodized oil than for any other group. In this non-viral gene transfer system, iodized oil emulsion is commonly used in HCC chemoembolization. The embolization effect, together with the lipophylic characteristics of this substance, may improve the specific transduction in hypervascular HCC. Because the iodized oil emulsion slows the blood flow, the gene complex will remain within the tumor vasculature for an extended period of time. It may increase the tumor selectivity and decrease the systemic exposure of gene therapy. Iodized oil emulsion can also have an additional antitumor effect by direct embolization of the hepatomas. The addition of the contrast agent iopamidol was used as the aqueous phase for the iodized oil emulsion to adjust the specific gravity of the inner aqueous phase so that it was equivalent to that of the iodized oil.

Shiba *et al*. (31) injected adenovirus vector expressing the herpes simplex virus thymidine kinase (AxCAHSVtk) and iodized oil esters (IOEs) or AxCAHSVtk alone through hepatic artery after HCC was induced in rats with diethylnitrosamine and phenobarbita. Then on postoperative days 2, 4 and 6, gancyclovir was injected into the peritoneum. The results showed liver dysfunction one week after gene therapy in the AxCAHSVtk with IOEs group was lower than in the AxCAHSVtk alone group, however, the survival rate was not significantly different. These results suggested that arterial injection of AxCAHSVtk with IOEs plus GCV could provide cancer-selective gene transfer to HCC and cause less liver dysfunction in surrounding normal tissue, but not improve the prognosis.

Stanford University interventional radiology group introduced a non-viral gene vector composed of iopamidol, protamine and ethiodized oil reagents (VIPER) for IA delivery (32). Through *in vitro* experiments, VIPER was compared to the most common used non-viral vector, cationic lipid FuGENE, and the most commonly used viral vector, adenovirus. The results showed a better selectivity for VIPER ($p<0.001$). Maximum transfection was achieved by using the optimized VIPER condition (50:1 protamine:DNA, 33% iopamidol, 2% ethiodized oil), with more than one order of magnitude selectivity for hepatoma cells compared with hepatocytes. Through *in vivo* experiments 10^6 Morris

hepatoma cells (McA-RH7777) were implanted to the left lobe of Buffalo rat. 13 days after implantaion positron emission tomography (PET) and ultrasound (US) were performed to confirm tumor implantation was successful. Then IA delivery was performed with the VIPER. The results were also promising. Altogether, IA delivery may have huge potential in clinical gene therapy for HCC.

Intra-tumoral injection. IT gene delivery is a local delivery route widely used in pre-clinical studies (33-35). Berraondo *et al*. evaluated the influence of route of administration on adeno-associated viruses (rAAV)-mediated liver transduction by comparing levels of luciferase expression in the livers of mice after injection of rAAV serotype 2, by applying three different routes of administration: IV, IP and IT injection (34). The analysis of transgene expression in mice, given IT injections, showed the highest luciferase expression value ($p<0.05$) in comparison with mice given IV and IP injections. However, after IT administration an inhomogeneous distribution was observed, with some areas highly transduced, and other areas showing virtually no transduced HCC cells. While only up to 30% of the tumor cells could be transduced by this technique, this proved to be sufficient for effective gene therapy using suicide genes exhibiting a strong bystander effect, or using immunomodulatory genes (36, 37-40). The IT image-guided technique is feasible in clinical practice. Apart from gene therapy, this direct percutaneous technique is routinely used to destroy intrahepatic tumor lesions (*e.g*. ablation, ethanol injection). It could, thus, be easily deployed for an intra-tumoral approach of gene therapy.

Although IT injection seems to be an approach with future potential, this strategy is not without limitations. Firstly, systemic leakage is difficult to avoid, resulting in extra-tumoral transduction, particularly in the normal hepatocytes scattered throughout the liver parenchyma. This extra-tumoral transduction is most likely a result of re-circulating vectors being drained into the circulation from the injected tumor sites. As HCCs are highly vascularized, direct injection of HCCs with a gene/vector complex could result in a substantial number of transduced normal hepatocytes (41). Secondly, IT injection is not feasible in all patients, particularly not in those with multiple tumor *foci*. Generally, image-guided, direct percutaneous techniques are not performed in patients who have more than 3 intrahepatic *foci* (42). Thirdly, this type of strategy has limited therapeutic benefit for small, distant metastases because it is a local therapy. And fourthly, at present, IT gene delivery could not provide a better response than percutaneous ablation, which is routinely used in the clinic today. All the above limit the usage of this gene delivery route in clinical practice.

Intra-portal injection. Although the study from Konno *et al*. indicated that HCC is mainly supplied by the hepatic artery, it is still believed that hepatomas, especially at the border zone of nodules, may as well receive blood supply from the portal vein (27, 43, 44). In some studies gene therapy was performed by IP administration and interesting findings were reported (45-47).

Lai Yin Tse *et al*. (45) tested the anti-tumor activity of adenoassociated virus (AAV) vector encoding kallistatin (an angiogenesis inhibitor which can exhibit anti-tumor activity) by IP administration. In their experiment, Hep3B cells of human origin (1×10^6) were injected into the upper left lobe of the liver in BALB/c nude mice, followed by IP transfusion of 3×10^{11} particles of rAAVkallistatin. Empty AAV, PBS and rAAV-EGFP served as controls. The mean size of tumors in livers 5 weeks after inoculation in mice treated with rAAV-kallistatin was significantly smaller than that in empty AAV or PBS-treated mice (by 70% and 66%, respectively, $p<0.05$ for both). There was no significant difference in tumor sizes between rAAV-EGFP, empty AAV and PBS-treated mice.

Hiraoka *et al*. (46) established multifocal hepatic tumors in syngeneic mice with murine CT26 colorectal cancer cells expressing firefly luciferase (CT26.FLuc). They infused Replication-competent retrovirus (RCR) vectors carrying the yeast cytosine deaminase (CD) gene *via* IP administration. Fourteen days after locoregional infusion, systemic administration of 5-fluorouracil resulted in significant inhibition of bioluminescent signals in mice whose tumors had been infected with RCR, but not in control mice. Notably, there was no detectable RCR vector spread to normal liver or bone marrow by quantitative PCR analysis.

Interestingly there were some studies which showed that with eligible parameters US exposure and systemic delivery of US contrast agent (microbubbles), gene expression could be enhanced by IP adminstration of plasmid DNA in the liver (48-49). Shen *et al*. systematically explored the use of microbubbles (MBs) and US exposure to improve gene transfer into the mouse liver (48). They delivered 10% MBs intraveneous and plasmid DNA into the mouse liver by IP administration. Co-presentation of 1-MHz US at a peak-negative pressure of 3 MPa exposures significantly increased luciferase gene expression with pDNA delivered by IP. The mean gene expression was 85-fold greater than that in the negative controls which without MBs and US exposure.

These studies demonstrated the potential of the IP administration route. However, because: (a) it is difficult to avoid the transduction of normal hepatocytes, which will aggravate the impairment of the liver function; (b) it has more restrictive limitations with regard to the size of the gene/vector complex to avoid occlusion of the portal vein (50); (c) in clinical practice, IP delivery probably has more technical pitfalls than other routes, the IP administration route is now mainly considered as an alternative route.

Intra-biliary injection. In theory, IB gene delivery could play an important role in the treatment of HCC, complicated with

bile duct involvement. The bile duct route provides some distinct advantages: (a), gene/vector complexes avoid the Kupffer cells and the interaction with blood components; and (b), because the biliary flow is very slow, the complexes are incubated in a relatively more static condition, which could facilitate gene transfer to tumor cells. Overall, the IB-infused complexes stand a better chance to be taken up by the targeted cells.

Pre-clinical studies demonstrated that gene complexes showed a high transduction rate *via* IB delivery. Meanwhile, no apparent complications were observed (51-53). Kuriyama *et al*. (53) administered recombinant adenovirus carrying a reporter lacZ gene retrogradely into the common bile duct of rats and evaluated the transduction efficiency of the lacZ gene in the liver histochemically by X-gal staining, and quantitatively by a chemiluminescent reporter gene assay. Although transgene expression induced by intrabiliary adenoviral administration was observed predominantly at periportal areas, a considerable number of cells expressing the transgene were detectable even in lobular and centrilobular areas. Furthermore, histochemical analysis revealed that intrabiliary adenoviral administration resulted in gene transfer into hepatocytes, but not into biliary epithelial cells. The biochemical analyses revealed that hepatic damage caused by intrabiliary adenoviral administration was not substantial. However, one of the major concerns relevant to this route is the interaction with bile and pancreatic juice. The effect of bile and pancreatic juice on gene delivery has been studied. Xie *et al*. showed that bile and pancreatic juice may affect certain gene vectors such as lipids. It was suggested that if the common bile duct is flushed before the delivery, transduction efficiency may be further improved (54).

Technically, bile duct injection can be easily adopted in the clinical setting through endoscopic retrograde cholangio-pancreatography (ERCP), a routine bile duct canulation procedure (55). Therefore, targeted gene delivery through the bile duct has some potential for treating HCC complicated with bile duct involvement.

Intra-splenic injection. IS injection is similar to the IP approach: both techniques consist of injecting the gene/vector complex into the portal circulation (45). The main difference with IP delivery is that IS injections may stimulate the immune system. The spleen is one of the most important lymphoid organs involved in the initiation of immune responses. As we learned from experience with cell transplantations, IS transplantation of some interleukin gene-modified cells is able to effectively activate immune functions (56). Moreover, it has been reported that efficient IS gene transfer could be achieved by *in vivo* electroporation without impairment or over-activation of immune response (57).

Studies on this subject showed some potential (45, 58-59). Zhang *et al*. (59) found that IS injection of adeno-associated virus (rAAV)-mediated anti-angiogenic gene could significantly inhibit tumor growth. They pre-delivered the rAAV-3TSR (the anti-angiogenic domain of thrombospondin-1) particles (10^{11}) in 100 μl *via* IS to immuno-compromised mice. Four weeks after gene/vector complex administration, they implanted cancer cells into the mouse pancreas. One month later they found the average tumor volume had decreased by 45.7% ($p<0.01$ *vs*. control).

However, regardless of these promising results this technique is technically complicated and suffers high-risk for complications. It is too early to apply this technique in clinical studies.

Other injection techniques. Last but not least there are some other delivery methods for gene therapy in HCC. One such technique is called the 'asanguineous liver in ischemia-hyperpressure procedure'. The liver is completely excluded from systemic circulation before the gene/vector complex is injected under high pressure in the infrahepatic vena cava. This approach has two main advantages. Firstly, the injection of gene/vector complex after vascular exclusion of the liver from the systemic circulation creates a retention of the gene/vector complex and concentrates the gene/vector complex in the liver, decreasing extrahepatic dissemination. Secondly, the liver sinusoidal endothelium contains fenestrations of 100 to 150 nm in diameter. Hyperpressure may force the gene/vector complex which is bigger than this criterion to cross the endothelial barrier.

Dariel *et al*. injected 2×10^8 infectious particles of lentiviral vectors encoding the green fluorescent protein marker gene in adult rats, under control of the liver-specific promoter transthyretin (60, 61). In the control group, gene delivery was performed by inflow IP injection. In the surgical group, the liver was completely excluded from the systemic circulation before viral injection in the intrahepatic *vena cava*. The results showed that short-term transduction efficiency was 14.35% in the surgical group compared to 0.39% in the control group ($p=0.016$).

The Ideal Delivery Technique

Ultimately, requirements for the ideal delivery method include safety, minimal invasiveness, and repeatability, along with allowing increased concentrations in the target zones, with homogeneity of expression and low systemic exposure (9). Among the various potential administration techniques for gene delivery in HCC, as discussed in this review, each approach has its own characteristics, summarized in Table I. Regarding the question on which administration technique for gene delivery in HCC is the best, despite the fact that different experimental protocols and different gene/vector complexes were used, controversial and contradictory results were so far obtained. However here is one conclusion:

Table I. *The relative merits of each gene delivery technique.*

Delivery technique	Systemic toxicity	Transduction rate	Degree of procedure difficulty	Procedure safety	Difficulty in clinical application
Intravenous	+++	+	+	+++	+++
Intra-arterial	+	+++	++	++	+
Intra-tumoral	++	++	++	++	+
Intra-portal	++	++	+++	++	++
Intra-biliary	+	++	+++	++	++
Intra-splenic	++	++	+++	+	+++

+++, High toxicity; ++, moderate toxicity, +, low toxicity. The intra-arterial approach seems to score best.

locoregional liver-directed approach seems to have the better trade-off between efficacy and toxicity than IV gene delivery. The unique anatomic features of the liver facilitate regional gene therapy approaches for unresectable hepatic metastases (62). The advantages of localized gene/vector delivery are obvious, such as induction of high-level expression *in situ* to achieve effective antitumor activity nearby or within tumor cells, and reducing the possibility of side-effects compared to a systemic delivery approach. Therefore, interventional techniques and gene therapy could be a good marriage; interventional techniques could provide the minimum invasive of both percutaneous and intravascular to reach the goal of locoregional gene delivery. During the locoregional delivery techniques, due to the unique blood supply of HCC, the IA delivery technique seems to provide the best balance between systemic toxicity, transduction rate, degree of procedure difficulty, procedure safety and difficulty in clinical application. However, this does not necessarily mean that IA is the best option in every presented case. The choice of the modality should be based on the clinical setting and the gene/vectors complex. For a specific clinical setting, such as HCC patients with systemic metastasis, systemic delivery with tumor-specific and a high expression gene is needed instead of locoregional delivery; HCC with cholangio cancer embolus, IB delivery seems to have a better potential. Regarding gene/vectors complexes, in order to let the delivery approach exert a maximum effect, one needs to choose the most suitable complex according to its specific features in relation to the delivery approach. For example, as we have discussed before, ethiodized oil reagents is a preferable vector for IA delivery.

Conclusion

The relative merits of each delivery technique for gene therapy in HCC were herein reviewed. Each delivery approach has its own characteristics and faces certain hurdles when applied to gene therapy in HCC. The IA gene delivery with ethiodized oil reagents seems to have a great potential, however, choosing for an ideal gene delivery approach should be still based on the clinical setting and the used gene/vector complex.

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