

RNA INTERFERENCE (RNAi) AS A PROMISING GENE THERAPY FOR HEPATOCELLULAR CARCINOMA

Shaymaa M.M. Yahya, PhD

Hormones Department, National Research Center, 12622, El-Tahrir Street Dokki Cairo- Egypt,
National Research Center, e- mail: yahshay10@yahoo.com

Abstract

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide. HCC is the fifth most common cancer and the fourth leading cause of cancer mortality globally. The treatment of HCC is limited because of underlying cirrhosis and a high rate of recurrence. RNAi is becoming a new tool in the diagnosis and treatment of diseases and new advances to prediction of the disease outcome. Having this concept, the man-made RNAi manufacturing has got a powerful market place in the interest of pharmaceutical companies. Because of the effectiveness and specificity in gene silencing, RNAi is expected to be applicable to gene therapies for hard-to-cure diseases. Both siRNA and shRNA induce cleavage and degradation of RNA molecules in a sequence-specific manner but has no effect on genes with unrelated sequences. Thus, RNAi presents itself as a promising yet novel therapeutic modality that is applicable against many cancers including liver cancers. Many cancer regulating genes were targets for RNAi (RNA silencing). One study aimed to analyze the effect of silencing the pituitary tumor transforming gene (PTTG) family on hepatocarcinogenesis. Other studies aimed at silencing the serine protease urokinase-type plasminogen activator (u-PA). A further study aimed at developing RNAi-based HCC therapy, assessed inhibition of function of human gankyrin gene product (p28GANK). RNAi-based approaches to the inhibition of vascularization of tumors have recently received attention. Other genes such as epidermal growth factor, BCL-2, and transforming growth factor- β were targets for RNAi. Use of RNAi technology to treat established HCC faces major difficulties. These include identification of optimal targets, efficient and safe delivery of RNAi sequences, and limitation of unintended off target effects. HCC also often presents as a disseminated cancer and safe delivery of RNAi effectors to all malignant cells will require improvement of currently available vectors.

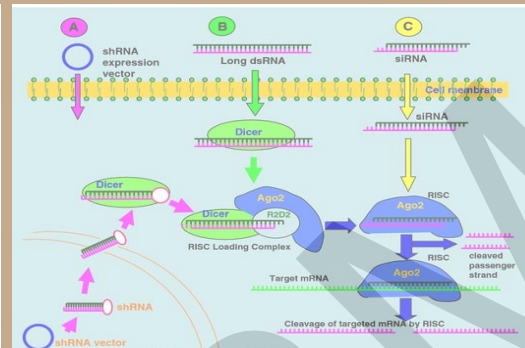


Fig (1): Mechanism of Specific Gene Silencing

Mechanism of Action

It is generally accepted that the RNAi cascade is initiated when the host cell encounters a long dsRNA from a virus or endogenous source. These long dsRNAs are cleaved into shorter dsRNA segments (siRNA) by the Dicer protein, ribonuclease III type protein. The RNA segments are 19–22 nt containing 5' phosphate and 3' overhangs with 3' hydroxyl termini in both the strands. These siRNA fragments are then incorporated into a large protein assembly, called the RNA-induced silencing complex, (RISC), wherein the sense strand of siRNA is removed by a helicase associated with the RISC. The RISC with the antisense strand specifically cleaves mRNA which has a complementary sequence to the antisense strand. The cleaved mRNA is then degraded immediately in the processing body (Fig 1).

In mammalian cells, dsRNA molecules, including siRNA and short hairpin RNA (shRNA) transcribed from a polymerase III promoter of a DNA fragment can efficiently induce RNAi activity. shRNA exported to the cytoplasm from the nucleus via Exportin-5 and the GTP-bound form of its cofactor Ran, utilizes the RNAi cascade in the same fashion as siRNA.

Cellular Oncogenic Sequences

As the molecular mechanism of hepatocarcinogenesis becomes better understood, so the number of potential targets that can be inhibited using RNAi improves. There are many oncogenes that have been described, which are implicated in the disruption of control of normal hepatocyte proliferation. Although this is encouraging, the targeting of specific cellular sequences is hampered by two main factors: (1) heterogeneity of gene expression in liver cancer cells from different sources, and (2) difficulty of achieving sufficient transfer of RNAi effectors to be of therapeutic benefit against the malignancy. The possibilities for therapeutic application are nevertheless intriguing.

A recent study aimed to analyze the effect of silencing the pituitary tumor transforming gene (PTTG) family on hepatocarcinogenesis. PTTG is a recently discovered group of oncogenes that plays a role in the genesis of several types of cancer through effects on cell division, apoptosis and DNA repair. PTTG1, but not PTTG2 and PTTG3, is frequently over expressed in patient liver cancer tissue as well as in established HCC line. Infecting cells with a recombinant adenovirus expressing an anti PTTG1 RNAi effector depleted cells of PTTG1 and resulted in the activation of p53 with consequent increased p21 expression and apoptosis. Inhibition of tumor growth in a nude mouse xenograft model of HCC further supported the notion that PTTG1 is a good candidate for RNAi mediated HCC therapy.

Other studies aimed at silencing the serine protease urokinase-type plasminogen activator (u-PA) demonstrated similar proof of principle efficacy. Signalling through u-PA and its receptor (uPAR) have been implicated in cell invasion, survival, and metastasis of a variety of cancers. Silencing of u-PA using RNAi based approaches has been used successfully in tumor models of prostate cancer and gliomas. To assess efficacy of RNAi-based u-PA silencing on HCC, Salvi and colleagues demonstrated that stable expression of a shRNA effectively knocked down u-PA. Moreover, silencing of u-PA resulted in attenuation of malignancy associated cellular properties, such as migration, invasion and proliferation

EGF (epidermal growth factor) is overexpressed and dysregulated in many solid tumors. (EGF), play critical roles in proliferation, apoptosis, angiogenesis, and metastasis. Their high expression is believed to mitigate the effectiveness of taxane chemotherapy by inhibiting cell division and apoptosis. Reports of inhibition of EGFR with tyrosine kinase inhibitors (TKI) [e.g. gefitinib (Iressa)] and monoclonal antibodies (e.g. cetuximab) have demonstrated that silencing of receptor activity increases chemosensitization of tumor cells. Targeting EGFR as well as other members of the human EGFR (HER) family has proven successful (Dickerson, 2010).

A further study aimed at developing RNAi-based HCC therapy, assessed inhibition of function of human gankyrin gene product (p28GANK). This novel oncogenic protein is ubiquitously over expressed in HCC and plays a role in cell cycle progression in normal hepatocytes and liver regeneration.

Challenges facing siRNA therapy

Although siRNAs offer several advantages as potential new drugs, there are challenges to be overcome in future research. These challenges include:

The 'off-target' effect: This is the inhibition of a gene, the expression of which should not be targeted, because the gene shares partial homology with the siRNA. To avoid this issue, the design and selection of potent siRNAs should be carefully performed. The basic parameters for choosing siRNAs involve consideration of internal repeated sequences, secondary structure, GC content, base preference at specific positions in the sense strand, and appropriate siRNA length (19–22 bps).

Innate immune response activation: Duplex RNA within cells is sensed as unwanted gene activity and may result in unintended harmful effects caused by activation of inflammatory cytokines and the interferon (IFN) response. Stimulation of cytoplasmic pattern recognition receptors, such as dsRNA dependent protein kinase (PKR), retinoic acid inducible gene- α (RIG- α) and Toll-like receptors (TLRs), leads to a cascade of events, which culminates in activation of transcription factors such as NF- κ B, IRF3 and IRF7.

Optimizing delivery vectors: One of the most difficult challenges impeding the advancement of RNAi-based HCC therapy is efficient and safe delivery of effector sequences. Ideally, vectors should deliver silencing molecules selectively to most if not all the malignant hepatocytes. Some trials have been done to optimize siRNA delivery for in vivo applications:

Chemical modification

To enhance the stability of siRNA for prolonged circulation in vivo, chemical modification of siRNA has been attempted. Various positions within the siRNA duplex have been chemically replaced or modified to provide nuclease resistance. One of the common approaches is replacement of the phosphodiester (PO4) group with phosphothioate (PS) at the 3'-end, the introduction of an O-methyl group (2'-O-Me), a fluoro (2'-F) group, or a 2-methoxyethyl (2'-O-MOE) group

Lipid-based siRNA delivery

Various lipid-based delivery systems have been developed for in vivo application of siRNA. Lipid-based systems include liposomes, micelles, emulsions, and solid lipid nanoparticles. For the delivery of siRNA using lipid-based systems, lipid composition, drug-to-lipid ratio, particle size, and the manufacturing process should be optimized.

Conjugates and targeted delivery of siRNA

For enhanced delivery, siRNA has been conjugated with various materials such as functional peptides, lipophilic molecules, and aptamers.

• Recombinant adenoviruses and adeno-associated viruses (AAVs):

They are capable of transducing liver cells with high efficiency and have been used successfully to deliver sequences that silence HBV or HCV gene expression.

Conclusion:

RNAi-based approaches for HCC treatment have recently received attention. However, these approaches faces major difficulties. These include identification of optimal targets, efficient and safe delivery of RNAi sequences, and limitation of unintended off target effects. HCC also often presents as a disseminated cancer and safe delivery of RNAi effectors to all malignant cells will require improvement of currently available vectors.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide, and its incidence continues to increase in the United States and Europe due to the spread of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection (Tanaka et al., 2008). HCC is the fifth most common cancer and the fourth leading cause of cancer mortality globally. HCC incidence has doubled in Egypt in the past 10 years (Lyer et al., 2009). The treatment of HCC is limited because of underlying cirrhosis and a high rate of recurrence; the cumulative 5-year survival rate is 53.4% with hepatic resection, 42.0% with local ablation therapy, and 22.6% with transcatheter arterial embolization in Japan. Radio-frequency ablation therapy has been recently introduced and has improved the survival rate. Nevertheless, even in small HCC, the 4-year survival rate is only 74%. The recurrence and prognosis of HCC is highly dependent on tumor extension and liver function. (Tanaka et al., 2008).

RNA interference (RNAi)

RNAi is becoming a new tool in the diagnosis and treatment of diseases and new advances to prediction of the disease outcome. Having this concept, the man-made RNAi manufacturing has got a powerful market place in the interest of pharmaceutical companies. Because of the effectiveness and specificity in gene silencing, siRNAs and shRNAs are expected to be applicable to gene therapies for hard-to-cure diseases, such as HIV infection (Coburn and Cullen, 2002), cancers (Scherr et al., 2003), and certain genetic disorders (Miller et al., 2004). Both siRNA and shRNA induce cleavage and degradation of RNA molecules in a sequence-specific manner (Li et al., 2002). RNA interference (RNAi) occurs in a variety of organisms, including *Caenorhabditis elegans* (Fire et al., 1998), *Trypanosoma brucei* (Wang et al., 2000), plants (Vaucher et al., 2000), *Drosophila* (Kavi et al., 2005), and mouse embryos (Svoboda et al., 2000). In most of these organisms, the injection of a double-stranded RNA (dsRNA) longer than 500 bp specifically suppresses the expression of the gene with the corresponding DNA sequence, but has no effect on genes with unrelated sequences.