Molecular Biology: Open Access
ISSN: 2168-9547

Bhadra et al., Mol Biol 2016, 5:2
DOI: 10.4172/2168-9547.1000158

Short Commentary Open Access

Get Well in the RNAi Way-RNAi, A Billion Dollar Baby in Therapy

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Abstract

Disease therapy based on genetic materials is now a reality. The technique of gene silencing also called as RNA interference (RNAi) keeps our hopes alive. RNAi based drugs have now advanced steps closer towards clinical trials. The powerful *in-vivo* RNAi machinery and its delicate factors apprehend that RNAi-dependent therapies might create a billion dollar business against the pathogenic organisms and diseases for which treatment options are currently restricted conventionally. Recent years have highlighted both the promises and challenges in the delivery of different RNAi therapeutics. Apart from the delivery, the design, stability and degradation of RNAi based effective molecules appear to be the major lime light to challenge the conventional drug safety concerns and ensures to be the most powerful gene recovery in future which may execute a billion dollar business hope.

Introduction

The blueprint of the human genome sequence, once announced in June 2000, has brought fresh air for modern medicines whose revolution has been overdue for a long time. But now enthusiasts believe that gene therapy and genetic material dependent treatments are advancing to transform conventional clinical practices. It is indeed a fantasy yet frustrating to cure a disease from its genetic origins, by repairing the defective human DNA or by destroying the parasitic DNA in human cells that frequently enter via infectious microbes particularly the air-borne viruses, jumping genetic elements etc.

RNA interference or RNAi is a remarkable process where small double-stranded duplexes silence homologous genes. Although first hint about this inducible silencing process was noticed few years ago, the actual quantum leap in perception of the phenomena came from two landmark papers by Pal-Bhadra, Fire and his colleagues [1,2]. Since then, the other units of the RNAi machinery has been revealed in a startling rate, although the picture is not yet complete.

RNAi is an internal gene silencing mechanism, manifesting almost in all eukaryotic cells, by which short double-stranded RNA molecules (typically 22bp) bring about inhibition of translation by the mRNAs degradation after pairing due to sequence complementarity [3-5]. Small RNAs including the siRNAs and microRNAs (miRNAs),are formed from ineffective hairpin pairing of RNA structures natively produced in the genome [6]. The mRNAs which are completely complementary are cleaved in a sequence-specific manner by RNAi effector molecules to implement gene silencing along with the repression of translation due to the degradation of transcript.

RNA-Modification Therapy

The RNA-modification therapy gives the essence of the whole idea of genetic medication or gene therapy for treating genetic disorders mainly by transferring genetic materials to make restitution for the aberrant phenotype for a definite genotype. The modification of mRNA to treat monogenic disorders is achieved primarily by four basic approaches: antisense oligonucleotides (ASO), RNAi, *trans*-splicing and ribozymes. The antisense oligonucleotides (ASO) are ssDNA sequences, 18–30 bases length. They bind to the mRNA based on sequence complementarity [7] and degrade them, resulting in reduced expression of the target gene. Its pre-clinical application, that affects the pre-mRNA processing has corrected the Duchenne muscular dystrophy (DMD) [8]. The main challenges for accomplishing the therapeutic role of RNAi underlies in improving its efficacy, specificity and, first and foremost, it's delivery. Another barrier in utilising RNAi as therapeutics is its likelihood of inducing the expression of type 1 interferon (IFN) responses [3,5] and saturation of innate RNAi pathway factors, owing to its dsRNAs (Figure 1). Newer strategies have been devised to deliver RNAi molecules with lipids or various vehicles that can transpass the membranes efficiently [3,5] because these molecules, besides having a short life span, are unable to easily traverse the cell membranes in human subjects. The hereditary disorders like the dominant-negative expansion of polyglutamine-spinal cerebellar ataxia type I and Huntington disease [9,10] comprise the two mouse models of neurodegerative disorders which can be treated by the combination of gene-transfer and RNAi .

In *trans*-splicing, a gene transfer vector delivers the sequence of the targeted pre-mRNA, post-alteration, exogenously to an independent pre-mRNA [11,12].

Ribozymes are RNA molecules possessing an enzymatic activity that catalyse phosphodiester bond cleavage at a site-specific way within the target molecule after the identification of specific RNA sequences [7,12,13].

The main hurdle in applying ribozymes to clinical trials is due to its inefficient delivery, instability and reduced efficacy. Ribozymes which were produced earlier could be delivered by exogenous means, but due to a low level of cellular uptake, if they were at all taken up, got degraded rapidly [7,13]. In laboratory studies, ribozymes have been applied *in-vitro* for the correction of familial amyloidotic polyneuropathy [14].

Advantages of RNAi Therapy

RNAi is actually beneficial as a therapeutic because it can precisely and effectively suppress the expression of genes of known sequence that can lead to a disease. In addition, it requires a comparatively less time for efficacy analysis. This along with the increased possibility of theoretical susceptibility of discovered pathogens to rapid targeting, has aroused great buzz about the competence of RNAi for treating a plethora of diseases. The first clinical trial of RNAi has treated the patients of wet, age-related macular degeneration (AMD) [15] and

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Received February 19, 2016; **Accepted** February 25, 2016; **Published** March 03, 2016

Citation: Bhadra U, Pal-Bhadra M, Das P (2016) Get Well in the RNAi Way-RNAi, A Billion Dollar Baby in Therapy. Mol Biol 5: 158. doi:10.4172/2168-9547.1000158

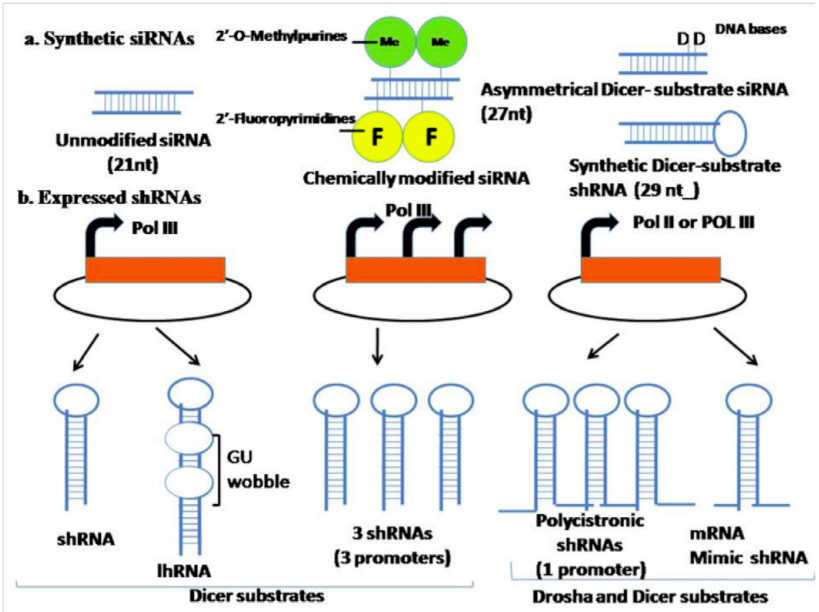

Figure 1: RNAi effector molecules.
a | Synthetic small interfering RNAs (siRNAs), produced by chemical modifications (middle panel)- such as 2′-Omethylpurines or 2′-fluoropyrimidines, added to increase stability are administered in vivo. Asymmetrical Dicer–substrate siRNAs having a blunt end bearing two DNA bases (D), and the other end bearing a 2-nt 3′ overhang. Dicer processes this blunt end to produce a single species of siRNA. Dicer also process longer synthetic short hairpin RNAs (shRNAs) as substrates.
b | Expression vectors drive high levels of shRNA expression from polymerase III (Pol III) promoters (left panel). Multiple Dicer-processed siRNA species generated by Long hairpin RNAs (lhRNAs), suggest that mammalian Dicer is processive .Multiple separate Pol III promoters can be used in one vector to drive expression of several different shRNAs (middle panel). Vectors carrying Pol II or Pol III promoters generate longer precursor RNAs, including polycistronic shRNA transcripts and microRNA (miRNA) mimics that are processed by both Drosha and Dicer (right panel).

respiratory syncytial virus (RSV) infection [16]. Preclinical strategies are being developed for other viral diseases [17,18], neurodegenerative disorders [19] and cancers [20]. RNAi can cure various diseases which are impossible to achieve using small molecule (chemical) drugs or protein-based therapeutics. RNAi-based drugs has been proved efficient in the treatment of various diseases, such as hypercholesterolemia [21,22], viral hepatitis [23] Huntington's disease [24,25] and cancer [26] in *in-vivo* studies in animal models.

RNAi in Cancer Treatment

The RNAi pathway has evolved to treat many areas including the CNS and cancer, because of its capacity to repress the endogenous gene expression.

RB1 and p53 result in apoptosis and reduction of cell proliferation in cancer cells. Gene products that inactivate RB1 and p53 are targeted by RNAi [27] while the anti-apoptotic proteins, such as Fas-associated death domain-like interleukin-1 beta-converting enzyme-like inhibitory protein (FLIP), Bcl-2, Bcl-xL and survivin, after being targeted, effectively control the activation of the proto-oncogene [20] siRNAs can silence the expression of the human telomerase RNA (hTER) template and heterogenous nuclear ribonucleoparticulate (hnRNP) A1/A2 proteins (which bind to telomeres) to check growth of cells and apoptosis, after targeting factors related with cellular senescence [28,29]. In the carcinogenesis pathway, the knockdown of targets associated with protein stability and degradation, and involved in the proteosome-dependent pathways has been used to inhibit the growth of tumour [20], VEGF is a pro-angiogenic gene that carries out neovascularisation. This gene after silencing [30], is found to prevent tumor growth in a xenograft model of mouse. Silencing of molecules involved in ECM degradation, such as MMP-9 and cathepsin B slows down the progression of tumor and in preclinical models of intracerebral glioma tumors, it leads to the total regression [31].

Immunosuppressive cytokines, such as interleukin 10 (IL-10) from many tumors facilitate the evasiveness of the tumour from the immune system. IL-10, when targeted by RNAi induces apoptosis [32].

RNAi therapies also target genes that provide resistance to chemotherapies used conventionally. Silencing the expression of multidrug resistance (MDR) genes, such as ABCB1 (MDR1 or P-glycoprotein), reverses the drug resistance and increases the susceptibility of glioma cancer cells to doxorubicin and vincristine [33].

Repair proteins like excision repair cross-complementing 1 (ERCC1) which makes cancer cells resistant to therapy-induced DNA damage are knocked down by RNAi-based therapies [20].

The vital cell-cycle proteins, such as kinesin spindle protein (KSP) and polo-like kinase 1 (PLK1), when treated with siRNA, exhibit impressive anti-tumor activity both in the hepatic and subcutaneous tumor model organisms [34]. The targeting of pleiotrophin, in mouse xenograft models of glioblastoma multiforme [35] reduces the growth of the tumor.

Inhibition of Protein kinase N3 (PKN3) reduces the lymph node metastases in orthotopic prostate cancer models [36]. The systemic administration of a siRNA-lipoplex , Atu027 against PKN3 in mice, rats and non-human primates, result in RNAi-mediated silencing of PKN3 expression inhibiting tumor growth and lymph node metastasis [37].

RNAi in Neurodegenerative Disorders

Most prominent RNAi delivery in the mouse brain are involved in treating spinocerebellar ataxia type 1 (SCA1) [38,39] a prominent class

of huntington disease. The SCA1 mutation generates polyglutamine (PolyQ) expansions, whose accumulation generates a toxic effect in neurological cells and is the ideal target for RNAi based knockdown. Such RNAi based targeting for reduction of Poly Q product was achieved by the AAV vehicle driven shRNA vectors even at a low transfection efficiency through the injection into the SCA1 mutant mouse brain.

An efficient lentivirus based delivery of siRNA in mouse models, targeted on superoxide dismutase I in case of the neurodegenerative disease, ALS or Amyotrophic lateral sclerosis lead to a stable and intense gene silencing again exemplifies the RNAi mediated therapy (Figure 2) [40]. It improves the motor nerve activity and delays the onset of diseased symptoms in defective phenotypes in mice. These two notable examples of viral delivery in model mice show strong potency of viral delivery strategies *in-vivo* devoid of toxic and viral related side effects.

Delivery and Uptake of siRNA Therapeutics

The widespread application of RNAi therapeutics is crippled by the inefficient cellular delivery and uptake of large negatively charged oligonucleotides. This suggests the possibility of targeted as well as localised uptake of these molecules.

Targeted Uptake

Targeted uptake is advantageous being potent for knockdown at a lower dosage in the target tissues without inflicting toxicity from the same in unexpected tissues. Antibodies with high-affinity or fragments [41-43], aptamers (nucleic acids selected for high-affinity binding) [44-46] or receptor ligands [21,47-50] binding to cell surface receptors can take up cell-specific siRNA. The siRNAs incorporated into lipid nanoparticles (LNPs) can be used for transfection in laboratory. The subcutaneous administration of siRNAs conjugated to trivalent *N*-acetylgalactosamine (GalNAc) [48,51-53] can be taken up by the hepatocytes through the hepatocyte-restricted asialoglycoprotein receptor (ASGPR) to knockdown the gene durably without increasing toxicity [54]. Hence, these conjugates are now being implemented in a Phase III study for treating rare genetic neurodegenerative diseases.

Non-Targeted Delivery

Administration of siRNA, which is not modified, results in silencing gene expression in various tissues. However, it is prone to degradation by nucleases in the serum and rapid renal excretion, can negatively affect the systemic delivery of unmodified siRNA, that reduces the efficiency of these drugs. siRNA conjugated with cholesterol or encapsulated in nanoparticles can enhance the stability of siRNA in the serum owing to chemical modification. In animal studies, there are non-viral and viral approaches, local and systemic administration, and multiple formulations (saline, lipids, and complexes or conjugates with small molecules,polymers, proteins and antibodies) have all been applied to achieve desired results.. Cell-specific siRNA delivery involves antibodies, ligands and aptamers. RNAi has been found effective to treat hypercholesterolemia and rheumatoid arthritis in mouse. RNAi has targeted exogenous genes in models of viral infection (hepatitis B virus (HBV), influenza virus, Ebola virus) and in tumor xenografts. Initial, RNAi-dependent trials aimed at the delivering siRNA locally or on objects, such as vascular endothelial growth factor (VEGF), for the treatment of the wet age-related macular degeneration and the respiratory syncytial virus (RSV) for the treatment of RSV infections but lately the efficient delivery of VEGF-specific siRNA is done by a direct injection into the vitreal cavity to the eye [55]. RNAi therapeutics through the direct delivery of siRNA into the lungs can treat RSV infection [56]. However, the fruitful and feasible RNAi-dependent therapeutics for many other diseases, particularly like cancer, require a more precise

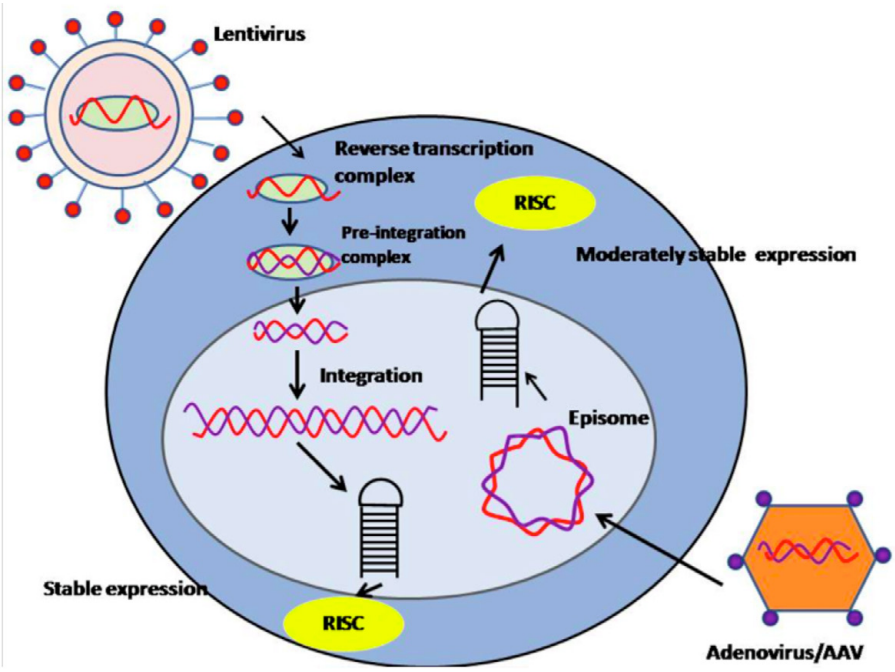

Figure 2: Viral delivery of short hairpin RNAs.
Lentiviral vectors are used to deliver therapeutic, short hairpin RNA (shRNA).These vectors express transgenes that integrate into the genome for a stable shRNA expression. This method is adopted in case of drug delivery to treat the neurodegenerative diseases like the ALS or **Amyotrophic lateral sclerosis**. Adenoviral and adeno-associated virus (AAV) vectors fail to integrate their transgenes into the genome and express shRNAs episomally for moderately stable levels of shRNA expression.

delivery to the tissues, remains yet to be realised.

Future Perspective

RNAi based therapies are now preceding towards clinical trials. Trials for HIV, hepatitis C virus vaccine and RNAi based medicine for Huntington disease has been started. The exponential progression of RNAi from basic technological invention to the application for human therapy is unprecedented. A higher concentration of promoter specific *shRNA* expression was found to suffer from toxic and fatal effects in mice. The target for RNAi based therapies is found to be applicable in a wide variety of cancers. But the main obstacle for this delivery is associated with the difference in metastasis for cells originating from initial tumor. The targeted siRNA can efficiently deliver in such cells provided metastatic populations are destroyed. In addition to these, different neurological disorders for example Huntington or ALS are also cured by RNAi. But the main issue is to deliver the RNAi medicine to the specific cell in the nervous tissues since the siRNA delivery vehicle cannot travel across the blood –brain barrier in the CNS. As an alternative, direct injection of siRNA in the brain has been proposed in mouse model so far. However, such approaches are bound to cause a definite obstruction in the human brains.

There are many potential reservations and problems for the use of RNAi-dependent therapy for curing human ailments. A more sophisticated delivery and an intricate as well as promising approach is required for a broad spectrum of human diseases. Going by the immense interest of RNAi based medicines, the years to come are likely to witness the extensive trials of these issues which can obviously improve the aspect of delivery, design and stability of RNAi mediated medicine to an ultimate level of perfection and efficacy.

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