Original Article

# Small Interfering RNA - Modern Approach for Intervening Pathological Conditions

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## Abstract

RNA interference (RNAi) refers to the inhibition of gene expression by small double- stranded RNA molecules. This technology can prove to be a breakthrough biological discovery of the decade as it has the potential to revolutionize the field of therapeutics. RNA interference (RNAi) through small interfering RNA (siRNA) is currently being evaluated for its efficacy to be used in therapeutics as well as prophylactic strategies. Many studies are being conducted across the globe to optimize the siRNA delivery systems (in terms of safe, stable and efficient delivery) in various disorders. There are a number of diseases such as autoimmune diseases, cancer associated pathological changes, bacterial and viral induced disorders, where RNAi pathway can be explored and RNAi technology can be used as a tool to intervene such abnormalities. This review is an effort to review latest advancements in the field of siRNA based therapy development and the pits and falls generally encountered in the use of this technology.

**Key words:** RNA interference, small interfering RNA, delivery mode.

## Remarkable Contributions in Field of Therapeutics By siRNA

Small interfering RNA (siRNA) induced gene silencing is one of the latest advancements in the field of molecular biology. siRNA belongs to a class of double-stranded RNA molecules, 20-25 nucleotides in length. These small nucleotide sequences can interfere with the expression of specific gene at a post transcriptional level, therefore causing a targeted disruption of a gene (Scherr *et al*., 2003). This technology is employed in research as well as in designing the new therapeutic strategies.

Although siRNA is widely used for studying a particular gene function and for elucidating signaling pathways however, therapeutics remains to be at the forefront of siRNA based technology (Ganesan *et al*., 2008). Clinical trials of RNAi- based therapies are being conducted. Targeted disruption of the culprit genes responsible for various pathological conditions resulting either from pathogenic interventions or due to abnormal functioning of body`s own immune system i.e autoimmunity is possible through siRNA (Hokaiwado *et al,*. 2008; McNamara *et al.,* 2006). Studies are being conducted in order to evaluate the use of siRNA technique in a wide range of pathologies e.g. in cardiovascular diseases (Tang *et al.*, 2007) various infections, cancers and autoimmunity in various *in vitro* as well as in vivo models, as well as in clinical trials (Eckstein *et al.,* 2010; Kumar *et al.,* 2008; Pruijn. 2006).

## Challenges Faced By the Researchers

The use of siRNA in therapeutics is a promising technology, however, there are some limitations offered by this state of art molecular tool (Shimaoka *et al.,* 2009). Two major issues generally faced by the researchers are; the stability of siRNA and the targeted delivery of siRNA to the desired cell population while maintaining a steady state environment.

Review of all the available data would be beyond the scope of this article, yet we have tried to focus on some recent, innovative advancements in the field of siRNA especially for therapeutic purposes and pits and fall related to it.

Direct administration of naked siRNA can silence the specific gene expression however due to the serum nucleases (RNA degrading enzymes), siRNA has relatively short half life (Lares *et al*., 2010; Poliseno *et al.,* 2004). In order to ensure maximum, stable and targeted incorporation of siRNA, various research groups employed various strategies. One of the developments is to couple siRNA with an immunoliposome. Zheung et al in 2009 was able to show the successful targeted delivery of siRNA to dendritic cells (DCs) (Zheng *et al.*, 2009). In an effort to generate tolerogenic dendritic cells (DCs), Zheung et al developed siRNA against CD40, a co-stimulatory molecule expressed by DCs. For this purpose they employed a polyethylene coated liposome containing the CD40 specific siRNA in order to suppress the CD40 gene expression. In order to ensure the targeted delivery of siRNA specifically to DCs they coupled the siRNA containing immunoliposomes with monoclonal antibody (NLDC-145) specifically targeting DC specific surface marker DEC-205. The final product encapsulated by an immunoliposome not only ensured the targeted delivery by means of the incorporated antibody but also protected the enclosed siRNA against RNA degrading enzymes (Zheung *et al.,*2009).

Nanoparticle (NP) technology is yet another exciting innovation which is currently being evaluated for its scientific utilization in various fields. Use of NPs in therapeutic and prophylactic purposes is also being

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evaluated (Dykxhoorn *et al.,* 2006). In face of various genomic approaches being employed for the treatment of cancers, siRNA based NPs offers a very promising tool for the down regulation of genes contributing towards cancer sustenance and progression (Aigner *et al.,* 2006). One such study specifically targeted the transferrin receptors expressed by tumor cells, which are typically up regulated in cancer cells. Transferrin receptors are responsible for providing cellular iron needed for cell survival (Muñoz *et al*., 2011) and hydroxylation of hypoxia-inducible factor-1α (HIF-α) by prolyl hydroxylases (PHD). The blocking of the transferrin receptors on cancer cells can be an effective way to prevent the cancer cell growth. Davis in 2009, conducted a study in which siRNA against transferin receptors was coupled with cyclodextrin containing polymer, polyethylene glycol (PEG) were assembled together into <100nm colloidal NPs (Davis. 2009). The pre-clinical and clinical use of such linear polymers has been demonstrated (Heidel. 2006). Results have shown the cell specific delivery and efficient gene silencing. Another similar study conducted by Shyh et al in 2008 (Li *et al.,* 2008) reported the use of NPs to deliver siRNA specifically to the tumor cells. Epidermal growth factor receptor (EGFR) is a cell surface receptor for the extracellular protein ligands and is involved in initiating signaling cascades, providing the necessary signal for cell proliferation, cell migration and cell adhesion (Koff *et al.,* 2008; Garrido *et al.,* 2011; Chan *et al.,* 2010). It is also involved in maintaining the functioning of innate immune response. Several studies have reported that mutations in EGFR can lead to various types of cancers (Quatrale *et al.,* 2011). Shyh *et al.,* in 2007 targeted this EGFR in lung cancer cells with specific siRNA and designed the assembly by utilizing the NP technology. He used a carrier DNA along with lipids and polyethylene glycol (so as to make the NP inert to the immune system) in the NP enclosing the siRNA. The innovative addition was that of protamine and anisamide ligand. Protamine enhances the efficiency of NP by helping the separation of enclosed siRNA from NPs at the targeted site of delivery. This is important as studies have reported that siRNA assemblies will not work unless the siRNA is separated efficiently from the assembly components at site of action (D. Reischl *et al.,* 2010). The anisamide ligand helps to enhance the siRNA uptake by the cells thereby increasing the efficiency of the RNA interference pathway. The effect of these NPs was studied in the NCI-H460 xenograft tumor (lung tumor) bearing mice. Tumor shrinkage was reported to be due to the enhanced tumor cell apoptosis.

Aptamers are a promising new class of targeting agents (Mayer *et al.,* 2011; Levy *et al.,* 2008; Ozpolat *et al.,* 2010; Li *et al.,* 2011). Aptamers are globular molecules formed of modified oligonucleotides. The high affinity, specificity and presence of resistance conferring modifications against serum and tissue nucleases makes aptamers a promising tool (Hicke *et al.,* 2000). An exciting study was conducted by James et al demonstrating the efficient use of aptamer- siRNA chimeric RNAs for the treatment of prostate cancers in xenograft model of prostate cancer (McNamara *et al.,* 2006). Aptamers were employed as a delivery system of siRNA targeting the genes involved in providing the necessary survival signal to the tumor cell for the tumor progression. The siRNA targeting the genes i.e. polo-like kinase 1 (PLK1) and BCL2 gene were coupled to the aptamers targeting a protein specifically expressed over the prostate cancer cells and tumor vascular endothelium i.e. Prostate-specific membrane antigen (PMSA). These aptamers-coupled siRNA were termed as the aptamer-siRNA chimeras. The aptamer-siRNA chimeras were evaluated in mouse xenograft prostrate cancer model and it was found that their binding was target specific and the targeted genes i.e. polo-like kinase 1 (PLK1) and BCL2 were significantly silenced hence providing a new gateway for the treatment of the prostate cancer.

Polyelectrolyte micelle complex based siRNA delivery system is another exciting proceeding towards the therapeutic utilization of siRNA in cancer (Parveen *et al.,* 2011). Sun et al reported the use of such siRNA delivery system for chemotherapeutic purposes. They utilized vascular endothelial growth factor (VEGF) siRNA and conjugated it with Polyethylene glycol. The PEG siRNA was further exposed to polyethlenimine (PEI) which resulted in spontaneous formation of nanoscale polyelectrolyte complex micelles. The final structure of the nanoparticle included an inner core of siRNA-Polyethylenimine and Polyelectrolyte micelle complex and this inner core was surrounded by polyethylene glycol sheet. Since angiogenesis has been implicated in metastasis and vascular endothelial growth factor (VEGF) has a direct role in cancer prognosis. Many VEGF blockers have been designed so far. In line with this, the in *vivo* VEGF suppression through the use of siRNA- based nanotechnology has been reported to cause reduced tumor size demonstrating the promising use of siRNA based therapy for treating cancer (Kim *et al.,* 2008).

Another utilization of siRNA is in the treatment of numerous inflammatory disorders such as those induced by tumor necrosis factor alpha (TNF-α). The major contribution in the pathogenesis of such disorder is played by macrophages and microglia cells (Glass *et al.,* 2010; Geissmann *et al.,* 2010). Sang et al in 2010 attempted to treat such a condition. In this study, he utilized the siRNA approach to cause suppression of TNF-α in macrophage and microglia cells and then evaluated its effectiveness in pathological state progression. It was found that such an intervention significantly reduced lipopolysacharide (LPS) induced neuro-inflammation and neuronal apoptosis in *vivo* system. The composition of the strategy included short nicotinic acetylcholine receptor (AchR) binding peptide derived from the rabies virus glycoprotein (RVG) which was used as targeting legend because the macrophage and microglia cells express (AchR) receptor on their surface. This peptide was further used for fusion with the nona-D-arginine residues (RVG-9dR) to enable siRNA- binding. Later on it was found that RVG-9dR did the target specific delivery of siRNA to induce gene silencing in macrophages and microglia cells from wild type, but not AchR-deficient mice indicating the effectiveness of RVG-9Dr being a way to deliver the siRNA to macrophage and microglia and hence suppressing the TNF-α and

suppressing the neuroinflammatory disorder`s pathogenesis (Kim *et al.,* 2010).

Dendritic cells (DC) are known for their functional dichotomy and are thought to be a cell population which joins together the two arms of immunity i.e. the innate and adaptive immunity (Iwasaki *et al.,* 2010; Schenten *et al.,* 2011; Van *et al.,* 2007). DC has the potential to stimulate and discriminate the naive T cells into any one of these population i. e T helper (Th) 1, Th2 cells and the T-regulatory (T reg) cells (Steinman *et al.,* 2006; Maldonado *et al.,* 2001; Belz *et al.,* 2002; Mahnke *et al.,* 2002; Min *et al.,* 2003) . However, the type of T-cell population generated by DC is determined by the expression of co-stimulatory molecules as well as certain cytokines (Kubach *et al.,* 2005). Th2 cell is dependent on the stimulation of naive T cell by IL-10 (Tuettenberg *et al.,* 2009; Ronet *et al.,* 2010) whereas Th1 production occurs in response to IL-12 produced by DC (O'Garra *et al.,* 2009). IL-10 is also implicated in the production of T-reg population (Heo *et al.,* 2009). This capability of DC to produce different subsets of T cells has largely been explored for its therapeutic utilization. Studies have been conducted with an aim to generate DCs with a specific phenotype that may interfere with the disease progression. Jonathan et al utilized siRNA strategy to target IL-12 *p35* and showed that IL-12 p70 production in bone marrow derived DC is significantly suppressed upon exposure to LPS and tumor necrosis factor alpha (TNF-α) with simultaneous increase in IL-10 production. These DC were then cultured with allogenic T cells, enhanced Th2 response was observed. This also led to the suppression of allostimulatory function of DC. It was further supported by another study reporting that siRNA treatment has resulted in the production of Th2 response promoting DCs by exposing them to keyhole limpet hemocyanin (KLH). This proves that siRNA can be utilized to produce the desired type of DC which can further be manipulated for intervening the disease processes (Hill *et al.,* 2003).

After reviewing some important advancement in the field of RNAi based therapies, we can say that RNAi is a promising new therapeutic modality. siRNA designed against various culprit genes have proven to be a good therapeutic intervention in various in *vitro* and in *vivo* experiments. However, the safe and effective method to deliver siRNA remains challenging. As discussed already that in order to design a stable and targeted delivery system, various groups have employed various delivery system. Such RNAi-based immuno-therapies are mostly in clinical trials. The success rate of such clinical studies will determine the future of many and can bring a revolution in the treatment of diseases.

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