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# Review on: Short Hairpin Ribonucleic Acid (RNA)

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**Abstract**
RNA interference (RNAi), an accurate and potent gene silencing method, was first experimentally documented in 1998 in Caenorhabditis elegans by Fire et al. Subsequent RNAi studies have demonstrated that the clinical potential of synthetic small interfering RNAs (siRNAs), micro RNAs (miRNAs) and short hairpin RNAs (shRNAs) used in various types of diseases such as cancer, neurodegenerative disorders and other metabolic diseases. siRNAs generally range from 21 to 25 base pairs (bp) in length and have sequence-homology-driven gene-knockdown capability. RNAi offers researchers an effortless tool for investigating biological systems by selectively silencing genes. Nevertheless, this area shows a huge potential for the pharmaceutical industry around the globe. Interestingly, recent studies have shown that the small RNA molecules, either indigenously produced as miRNAs) or exogenously administered synthetic dsRNAs, could effectively activate a particular gene in a sequence specific manner instead of silencing it. This novel phenomenon has been termed 'RNA activation' (RNAa). In this review, we analyze these research findings and discussed the function and applications of siRNAs, miRNAs, and shRNAs.

**Keywords:** RNA interference, siRNA, miRNA, shRNA, Gene silencing.

## Introduction
It was well-established that the nematode that is Caenorhabditis elegans, a model genetic organism for studying the behaviour and development of humans (C. Kenyon et al., 1988), for which they were awarded the 2002 Nobel Prize for Physiology/Medicine. Recently, two key phenomenal discoveries emerging from C. elegans were the identification of inherent tiny, non-coding RNA genes, lin-4 and let7, which produce microRNAs (miRNAs) that regulate development and the establishment of RNA interference (RNAi) by long double stranded RNAs (dsRNAs). Fire and Mello demonstrated that 21- to 25-nucleotide short interfering RNAs (siRNAs) are the key effector molecules of RNAi in Caenorhabditis elegans (A. Fire et al., 1998). Conversely to siRNAs, miRNAs are derived from the processing of endogenously encoded short hairpin RNAs (shRNAs). In contrast, the exogenous siRNAs hypothetically represent perfect drugs for the specific blocking of unwanted or disease-causing gene products there is no disease affecting humans that does not have a genetic component, and RNAi sequences can be tailored to block just about any gene (L. Aagaard et al., 2007). These sequences make blocking possible by binding and then degrading the mRNA produced by the gene before that mRNA can start producing a harmful protein, which actually causes the illness. Ever since the Nobel Prize-winning discovery by Fire et al, in 1998, more than 12,000 articles and 1300 reviews related to RNAi technology have been published. In this review, I analyse these research findings and discuss the function and applications of RNAi.

## Discovery of shRNA:
At the first observation of gene silencing induced by RNA interference (RNAi) was back in early 1990s in plant system in the efforts to make the color of flowers more vivid, that's why researchers created transgenic plants with extra copies of pigment-making genes. To their surprise, instead to be more colorful, some of the transgenic flowers turned out to be white"co-suppressed" (A.R. van der Krol, et al., 1990). The transgenic copy, intended to

make more corresponding gene products. That is post transcriptional gene silencing (PTGS), was not limited to plants. Guo and Kemphues parallel work in C.elegans was carried on in 1995. They injected the antisense strand RNAs of certain endogenous gene into worms, with intension that those antisense strands may hybridized to corresponding gene and in turn block translation. Previous study showed that sense strand RNAs, which in fact the same as endogenous target mRNA itself were used as negative control. Shockingly, sense strand by itself was found to be as effective as antisense to suppress gene expression (S. Guo et al., 1995). But, this mystery was solved by Fire and Mello in 1998, when they tested the synergy effect of sense and antisense strand together. Then they found that double-stranded RNA (dsRNA) was at least ten-fold or perhaps a hundred fold more potent as a silence trigger than was sense or antisense alone. Furthermore, they pointed out the repression effect which is observed by Guo and Kemphues in 1995 was in fact caused by trace amount of double-stranded RNA contamination in those samples (A. Fire et al., 1998). After that the recent approach is used of a bacterial vector to obtain shRNA expression in cells. Recombinant Escherichia coli, containing a plasmid with shRNA, fed to mice can knockdown target gene expression in the intestinal epithelium. This approach was used in 2012 in clinical trials to try to treat patients with Familial Adenomatous Polyposis (C.J. Burnett et al, 2011). A variety of viral vectors can be used to obtain shRNA expression in cells including adeno associated viruses (AAVs), adenoviruses, and lentiviruses. With adeno associated viruses and adenoviruses, the genomes remain episomal. This is advantageous as insertional mutagenesis is avoided. It is disadvantageous in that the progeny of the cell will lose the virus quickly through cell division unless the cell divides very slowly. AAVs differ from adenoviruses in that the viral genes have been removed and they have diminished packing capacity. Lentiviruses integrate into sections of transcriptionally active chromatin and are thus passed on to progeny cells. With this approach it was demonstrate that there is increased of risk in insertional mutagenesis; However, the risk can be reduced by using an integrase-deficient lentivirus (A. Lombardo et al., 2007).

**Review of Literature**

RNA interference (RNAi), a target gene silenceing method in the basis of functions it's include various types. Here I describes some specific RNAi shortly but briefly describes only short hairpin RNAs (shRNAs).

**Micro RNA (miRNA)**

Micro-RNAs are a class of endogenous double stranded RNAs that exert their effects through the RNA interfering pathway. It's also proved by Grishok that miRNAs are a group of small non-coding RNA molecules produced by endogenously (MA. Grishok et al., 2000). More than a decade elapsed between the discovery of the first miRNA, Caenorhabditis elegans lin-4 (R.C Lee et al., 1993) and the achievement of at least a superficial understanding of the biosynthesis, processing and mode of action of this class of noncoding regulatory RNAs. From C. elegans to higher vertebrate's miRNAs can play an important functions by base-pairing to mRNAs to regulate the expression of a specific gene. It was reported that the number of miRNAs so far is in excess of 5300, several-fold as many as initial calculations, and thousands of predicted miRNAs are awaiting experimental confirmation. Through mechanisms that have yet to be discovered, miRNAs are transcribed by RNA polymerase (pol) II as long primary polyadenylated transcripts (pri-miRNAs) (Y. Lee et al., 2004). A previous study showed after that the pri-miRNA is recognized and cleaved at a specific processing site by the complex of RNase III enzyme and Drosha, and produce a miRNA precursor (pre-miRNA) of approximately 70–90 nucleotides (nt). The pre-miRNA has a 2-nt 3′ overhang at one end17. This distinctive structure is recognized by the Exportin-5–Ran-GTP heterodimer, and the pre-miRNAs is shuttled to the cytoplasm (R. Yi et al., 2003). It has been shown that only the miRNA precursor recognized and processed by Dicer into a mature miRNA, using the 3′ overhang as a guide for site-specific cleavage at the second processing site (D. Siolas, et al., 2005).

**Small Interfering RNA (siRNA)**

Investigators have assessed that fluorescent labeled siRNA has been used to trace the fate of delivered siRNA. A fluorescent label can either be tagged onto the 5′ end or the 3′ end of the siRNA. The Using a fluorescence resonance energy transfer (FRET)-based visualization method, the intact siRNA can be observed to be translocated into the nucleus within 15 min of the delivery and then disseminated in to the cytoplasm within the next 4 h both in intact and dissociated form. It was well known established that the initial accumulation of siRNA in the nuclei is similar to observations made on the behavior of antisense olignucleotides (J.P. Leonetti et al,. 1991). A previous study showed that Ago1 and Ago2 containing RISC were found in the cytoplasm and as well as in nucleus (S. Rudel,. et al,.2008). A recent study using fluorescence correlation spectroscopy and fluorescence cross-correlation spectroscopy (FCS/FCCS) to correlate the presence of siRNA with Ago2 protein indicated a shuttling of RISC between nucleus and cytoplasm. Importin 8 (Imp8) binds to all Ago proteins in a Ran-dependent manner, but independently of RNA (L. Weinmann et al, 2008). Knock down of Imp8 results in a shift of Ago2 from the nucleus to the cytoplasm without affecting the total quantity of Ago2. However, although Imp8 is not required for target mRNA cleavage it is necessary for Ago2 binding to miRNA targets. But in Caenorhabditis elegans, it was identified that the argonaute protein NRDE-3 is essential for binding nuclear RNAs and appears to interact with cytoplasmic siRNAs generated by RNA-dependent RNA polymerase (RdRP) followed by redistribution to the nucleus (S. Guang et al,. 2008).The steady-state nuclear distribution of siRNA was excluded from the nucleoplasm but found in the nucleolus region (T. Ohrt,. et al,. 2006). The cytoplasmic distribution of siRNA appears to be in the perinuclear region forming a ring-like pattern around the nucleus (Y.L. Chiu et al., 2004). The nucleolus and perinuclear regions are possibly the main site for RNAi. In case of Drosophila, double-stranded RNA-binding proteins (dsRBPs), such as R2D2 and Loquacious (Loqs), function in tandem with Dicer enzymes in RNAi. Dcr-1/Loqs and Dcr-2/R2D2 complexes generate microRNAs and small interfering RNAs, respectively.

It was suggested that in mammalian cells, only one Dicer gene has thus far been identified (E.P. Murchison et al.,

2005). Dicer is an integral component of the RNA interference pathway for human. Dicer processes pre-microRNA and double-strand RNA (dsRNA) to mature miRNA and siRNA, respectively, and transfers the processed products to the RISCE (E. Bernstein et al., 2001).Dicer is a multi-domain RNase III-related endonuclease responsible for processing dsRNA to siRNAs (S.M. Hammond et al., 2001). Dicer preferentially binds to the 5′ phosphate of 2 nt 3′ overhang and cleaves dsRNAs into 21 to 22 nucleotide siRNAs (M.A. Carmell et al,. 2004). Duplex siRNA in association with holo-RISC, composed of at least Ago-2, Dicer and TRBP, is identified as the RISC loading complex (RLC) (G.B. Robb et al., 2007). In the RLC, the two strands of the duplex are separated, resulting in the departure of the passenger strand (P.J. Leuschner et al., 2006). The passenger strand is cleaved by the RNase-H like activity of Ago-2, provided there are thermodynamically favorable conditions for passenger strand departure. This is referred to as the cleavage-dependent pathway (J.B. Preall et al., 2005). There is also a cleavage-independent by-pass pathway, in which the passenger strand with mismatches is induced to unwind and depart by an ATP dependent helicase activity (J.B. Preall et al., 2005). The RISC with single-stranded guide strand siRNA is then able to execute multiple rounds of RNA interference. ATP is not required for shRNA processing, RISC assembly, cleavage-dependent pathway, or multiple rounds of target-RNA cleavage (E. Maniataki et al., 2005). Single-stranded siRNA (containing 5′-phosphates) and pre-miRNA can be loaded on RISC, but not duplex siRNA (R.L. Boudreau et al., 2008).

**Short Hairpin RNA (shRNA)**

It was first demonstrate by Tuschl and colleagues, they showed that short RNA duplexes, designed to mimic the products of the Dicer enzyme, could trigger RNA interference in vitro in Drosophila embryo extracts (T. Tuschl et al., 1999). This observation was continuously extended to mammalian somatic cells by the help of both Tuschl and coworkers and by Fire and colleagues (N.J. Caplen et al., 2001), they showed that chemically synthesized siRNAs could induce gene silencing in a wide range of human and mouse cell lines. So the use of synthetic siRNAs in mammalian cells is quickly becoming a method of choice for probing gene function because it's transiently suppress the expression of target genes in mammalian cells.

**Vector choice for shRNAs**

It was established that the Dharmacon™ SMARTvector™ Lentiviral shRNA platform is an innovative system ideally suited for RNAi –mediated gene silencing. For this reason it's provide to the researcher with the most effective tools for deliver and expressing genetic content in their cells of interest. So, the platform utilizes the advanced design of SMARTvector Lentiviral shRNA and employs:

- A microRNA scaffold
- Enhanced rational designs
- Seven RNA polymerase II promoter options for experiments in a broad range of cell types
- Two fluorescent reporter options TurboGFP (tGFP), TurboRFP (tRFP) • Expanded depth of coverage per gene
- An option of ultra-high titer of $5 \times 10^9$ TU/mL

Additionally, the designs have been rigorously tested in an endogenous system and include a functional guarantee. The SMART vector work flow incorporates a unique tool, the SMART choice Promoter Selection Plate (Cat #SP 001000-01).This is a 96-well plate containing high-titer, transduction ready lentiviral particles derived from seven unique lentiviral shRNA constructs, each containing a different transcriptional promoter. The SMART choice Promoter Selection Plate allows the researcher to evaluate and then choose the vector configuration that is most effective for shRNA expression in the particular cells of interest. This semi-customizable approach to vector design provides greater flexibility, and more importantly, improved likelihood of experimental success relative to static systems.

**Invasion of shRNA**

A previous study showed that shRNAs, as opposed to siRNAs, are synthesized in the nucleus of cells, further processed and transported to the cytoplasm, and then incorporated into the RISC for activity (B.R. Cullen et al,. 2005). To be effective, the shRNA are designed to follow the rules predicated by the specifics of the cellular machinery and are presumably processed similar to the microRNA maturation pathways. That's why the studies on miRNAs synthesis and maturation has provided the groundwork for the synthesis of shRNA particularly the miRNA -30 based shRNAs. shRNA transcribed on the basis of promoters sequence that's why it's transcribe by either RNA polymerase II or III.It was well known established that the primary transcript generated from RNA polymerase II promoter contain a hairpin like stem-loop structure that is processed in the nucleus by a complex containing the RNase III enzyme Drosha and the double-stranded RNA-binding domain protein DGCR8 (Y. Lee et al.,2003).The complex measures the hairpin and allows precise processing of the long primary transcripts into individual shRNAs with a 2 nt 3′ overhang (H. Zhang et al., 2002 ). The processed primary transcript is the pre-shRNA molecule. It is transported to the cytoplasm by exportin 5, a Ran-GTP dependent mechanism (B.R. Cullen et al., 2004). In the cytoplasm the pre-shRNA is loaded onto another RNase III complex containing the RNase III enzyme Dicer and TRBP/PACT where the loop of the hairpin is processed off to form a double-stranded siRNA with2 nt 3′ overhangs (R. Yi et al,. 2008). The Dicer containing complex then coordinates loading onto the Ago2 protein containing RISC as described earlier for siRNA. Pre-shRNA has been found to be part of the RLC; thus, pre-shRNA may potentially directly associate with RLC rather than through a two steps process via a different Dicer/TRBP/PACT complex (M. Landthaler et al., 2008). After loading on to RLC and passenger strand departure; both siRNA and shRNA in the RISC, in principle, should behave the same.The argonaute family of proteins is the major component of RISC. Within the Argonaute family of proteins, only Ago2 contains the endonuclease activity necessary to cleave and release the passenger strand of the double-stranded stem (J.B. Preall et al,. 2005). The remaining three members of Argonaute family, Ago1, Ago3 and Ago4, which do not have identifiable endonuclease activity, are also assembled into RISC and presumably function through a cleavage-independent manner.

**Function of shRNA in to the cell**

Thus, RISC can be further classified as cleavage-dependent and cleavage-independent. The argonaute family of proteins in RISCs are not only involved in the loading of siRNA or miRNA, but also implicated in both transcriptional (targeting heterochromatin) and post-transcriptional gene silencing. Ago protein complexes loaded with passenger strand less siRNA or miRNA seeks out complementary target sites in mRNAs, where endonucleolytically active Ago-2 cleaves mRNA to initiate mRNA degradation (G. Hutvagner et al,. 2002). Other Ago protein containing complexes without endonucleolytic activity predominantly bind to partially complementary target sites located at the 3′ UTR for translation repression through mRNA sequestration in processing bodies (p-bodies) (D.T. Humphreys et al,. 2005). The detailed mechanism of mRNA sequestration in p-bodies and later release from p-bodies is still adebated issue; deadenylation of the target mRNA which leads to destabilization of the mRNA was also observed to occur in p-bodies (M.A. Valencia-Sanchez et al,. 2006.). Coimmunoprecipitation experiments determined that RISCs are also strongly associated with polyribosomes or the small subunit ribosomes (M. Landthaler et al., 2008) and Ago-2 (actually identified as elF2c2), strongly suggesting that RISC surveillance is compartmentalized with translational machinery of the cell. Details of the mechanism involving mRNA scanning and target mRNA identification are still largely unknown. Whatever the scanning or surveillance mechanism may be, once the target mRNA is identified, the target mRNA is either cleaved or conformationally changed following which both types of structures are routed to the p-body for either sequestration or degradation (M.A. Valencia-Sanchez et al,. 2006). The active siRNA or miRNA loaded complex is then released for additional rounds of gene silencing activity.

**Application**

Applications of RNAi both in vivo and in vitro are recently approach. RNAi has been used to generate model systems for in vivo study, to identify novel molecular targets and gene function in a genome, and to create a new niche for medical therapeutics (FY. Xie et al,, 2007). We have reviewed the therapeutic potential recently for synthetic siRNAs and shRNAs in various human diseases and disorders. Both shRNAs and siRNAs have been successfully tested in various disease models of animals, and I discuss some of those studies in this review in the following section.

**In Vivo Rnai In Oral Diseases**

1. **Nasopharyngeal Carcinoma** In hyaluronan receptor (CD44) gene expression by RNAi is the specific gene silencing in the carcinoma cell line (CNE-2L2) of nasopharyngeal resulted in profound reduction of malignant potential of the cells, including growth, in vitro colony formation and metastasis of tumors in nude mice ( Y. Shi et al., 2007a). In nude mice inhibition of tumor growth caused by directly injection of the adenoviruses harboring and producing siRNA to CD44 into the tumor inoculated with CNE-2L2 cells. Analysis the data of CD44 expression with the malignant activities of CNE-2L2 cells indicated a positive association and suggested an important therapeutic effect of direct introduction of siRNA to CD44 into some human solid tumors with high expression of the CD44 gene (Y. Shi et al., 2007a,).
2. **Head and Neck Cancer** It was identified that the genes, which is potentially serve as molecular therapeutic markers for human head and neck cancer, (YJ. Chen et al., 2007) which used for differential display analysis to compare the both the gene expression profiles of head and neck cancer cells and also in histopathologically normal epithelial cells. They identified the desmoglein 3, which is differentially expressed in both the protein & RNA levels. Consistent with the clinical findings, RNAi significantly inhibit the demoglein 3 and reduced the cell growth and colony formation 57-21% in head and neck cancer cell lines. Moreover, in vivo experiments of xenograft suggest that the administration of desmoglein 3-RNAi plasmid significantly inhibited tumor growth for 2 mos in BALB/C nude mice. For this findings of RNAi magnetize that the desmoglein 3 is a potential molecular target for development of adjuvant therapy for both head and neck cancer (YJ. Chen et al., 2007).
3. **Oral Squamous Cell Carcinoma** An F box protein that is kinase-interacting protein 2, which is required for the ubiquitination and compliant degradation of p27 in S-phase.In various cancers cells, reduced expression of p27 is frequently observed, including oral squamous cell carcinoma, due to an enhancement of its protein degradation. It was well known established that over expression of S phase kinase-interacting protein 2 was found in oral squamous cell carcinoma and inversely correlated with p27 expression (Y. Kudo et al., 2005b). A previous study showed that the S phase kinase-interacting protein 2 siRNA inhibited the cell proliferation of oral squamous carcinoma cells both in vivo and in vitro. So, those findings proved that siRNA-mediated gene silencing of S phase kinase-interacting protein 2 is a novel modality of cancer gene therapy for suppression of p27 down regulation. It suggest that the basal transcription of p53-inducible ribonucleotide reductase small-subunit 2 could be associated with the sensitivity for anticancer agents (S. Yanamoto et al., 2005). It has been shown that the p53-inducible ribonucleotide reductase small-subunit 2 may be a good molecular target, and it was reported that the RNAi targeting of p53 inducible ribonucleotide reductase small-subunit 2 would be useful for oral cancer gene therapy (S. Yanamoto et al., 2005).
4. **Tooth Development** Investigators have assessed the possibility of RNAi for used to investigate the roles of key genes involved in the development of tooth. It was established that viral-mediated RNAi knockdown of msh-like 1, homeobox, or distal-less homeobox 2 mRNAs in the dental mesenchyme, deficient for homeobox in mice, it was possible to reproduce the identical tooth phenotype seen both in msh-like 1 and distal-less homeobox 2 genes, respectively. It suggest that at the bud stage silencing of homeobox and msh-like 1 in the dental mesenchyme results in an arrest of tooth development, indicates a critical role for homeobox, msh-like 1 in tooth development.

**In Vitro Rnai In Oral Diseases**

It has been shown that Some of the recent RNAi studies in vitro can be potentially used for the treatment of dental disorders such as periodontal and ankylosis diseases. Periodontal-ligament-associated protein-1 (PLAP-1)/asporin is involved in chondrogenesis, and its involvement in the pathogenesis of osteoarthritis has been proved (S. Yamada et al., 2001). Over expression of asporin in mouse periodontal ligament-derived clone cells interfered with both naturally and bone morphogenetic protein-2-induced mineralization of the periodontal ligament cells. In contrast, knockdown of asporin transcript levels by RNAi enhanced bone morphogenetic protein-2-induced differentiation of periodontal ligament cells. These results suggest that asporin plays a specific role(s) in the periodontal ligament as a negative regulator of cytodifferentiation and mineralization, probably by regulating BMP-2 activity to prevent the periodontal ligament from developing non-physiological mineralization such as ankylosis (S. Yamada et al., 2007). Recently, Downloaded from jdr.sagepub.com used H413 epithelial cells derived from a human oral squamous cell carcinoma and 'knocked down' the CD24 protein by RNAi to cause about 90% reduction in the mRNA level. CD24 is a heavily glycosylated peptide molecule implicated in hematogenous metastasis of carcinomas (S. Schindelmann et al., 2002). This down-regulation of CD24 mRNA was associated with reduced E-cadherin expression and up-regulated expression of transcription factors such as snail, twist, and transforming growth factor-b3. Hence, it was concluded that CD24 could play an important role in modulating the expression of genes that regulate epithelial differentiation in the periodontium.

**In vivo RNAi in Acute and Chronic Inflammation**

A previous study showed that sepsis and allergy are very important diseases affecting in human beings (AJ. Melendez et al., 2007). Recently, it's demonstrate that specific gene silencing of the mouse sphingosine kinase 1 isoform results in the amelioration of C5a-induced acute peritonitis and allergic asthma (WQ. Lai et al.,) in mouse models of disease.

**In vivo RNAi in the Regulation of Splice Variants**

It was demonstrate that the abnormal regulation of splice variant expression of certain genes has been recognized as the reason for several genetic disorders in humans, including cancer (MA. Garcia-Blanco et al., 2004). The human growth hormone (hGH) gene contains 5 exons as well as 4 introns. Deduction of all 4 introns produces mRNA that encodes the full-length 22-kDa hGH protein. It has been shown that the exon-C-skipped mRNA encodes a 17.5-kDa protein which is linked to isolated hGH deficiency (IGHD) type II, an autosomal-dominant form of hGH deficiency (GHD). Therapies that specifically target the 17.5-kDa isoform might be useful in persons with IGHD II (JP. Monson et al., 2003). Certainly, RNAi has the potential to be an efficient alternative to current hGH replacement therapy, which has many side-effects, such as benign intracranial hypertension and insulin resistance (RC. Ryther et al., 2004).

**In vivo RNAi in Neurodegenerative Disorders**

RNAi knockdown of mutant SOD 1 slows amyotrophic lateral sclerosis (ALS) in animal models (Wang et al., 2008). A recently well-known study by Alnylam Pharmaceuticals, Inc. and collaborators from the University of Massachusetts Medical School and Massachusetts General Hospital suggest that chemically synthesized RNAi targeting the gene responsible for Huntington's disease provide a therapeutic benefit in an animal model of the human disease. The new preclinical study demonstrate that a single injection of a cholesterol conjugated-siRNA targeting huntingtin, the gene responsible for Huntington's disease, resulted in improved symptoms of disease in an animal model. These improved effects included reduction in neuronal pathology and an improvement in motor behavior. In preliminary studies, the RNAi therapy reduced expression of mutant huntingtin in the brain and sustained a benefit in motor behavior for at least one week. The RNAi therapy was found to be well-tolerated in the brain after direct CNS administration (DiFiglia et al., 2007).

**New About shRNA**

The ability of shRNA to provide specific, longlasting, gene silencing there has been great interest in using shRNA for gene therapy applications. Here some examples of shRNA-based therapies are discussed in below. Gradalis, Inc. developed the FANG vaccine, which is used in treatment of advanced cancers. FANG relies on a bi-functional shRNA (bishRNA) against the immunosuppressive transforming growth factors (TGF) β1 and β2. Autologous tumor cells were harvested from patients and a plasmid encoding the bi-functional shRNA and granulocytemacrophage colony-stimulating factor (GMCSF) was introduced ex vivo through electroporation. These cells were later irradiated and injected back into the patient. A previous phase 1 safety study demonstrated that the FANG vaccine was safe. It also showed that the vaccine did activate T-cells, as confirmed by gamma interferon enzyme linked immunospot assay (ELISPOT) positivity. Importantly, however the phase 1 study was made up of a "gag bag" of patients with different tumors, including colon cancer, thyroid cancer and ovarian cancer. Marina Biotech developed CEQ508 which is used to treat Familial Adenomatous Polyposis. CEQ508 uses a bacterial vector to deliver shRNA against βcatenin.Gradalis, Inc. developed bifunctional shRNASTMN1 (pbishRNA STMN1), which is used to treat advanced and/or metastatic cancers. This pbi-shRNA STMN1 is against stathmin 1 and is delivered intra tumorally through bilamellar invaginated vesicle (BIV) lipoplex (LP) technology. Several challenges typically confront shRNAbased therapeutics. The most significant challenge is delivery.shRNA is typically delivered through use of a vector, and although they are generally efficient, they pose significant safety concerns. In particular, viral based gene therapy approaches have proved dangerous in past clinical trials. In the first generation of retro viral gene therapy some patients treated with viral vectors for (wiskottAldrich syndrome) WAS developed acute Tcell leukaemia. This was determined to have been caused by viral vector insertion location. Potential oversaturation of RISC is also a problem. If the shRNA is expressed at levels that are too high the cell might not be able to correctly process the endogenous RNA which could cause significant problems. Another challenge is the possibility that the patient might mount an immune response against the therapy. Finally, there might be offtarget effects and the

shRNA could silence other unintended genes. In developing successful new shRNA based therapeutics, all of these challenges must be taken into account.

## Conclusion

Now it is possible to easily identify specific anti-proliferative genes for numerous cancer cell lines through the specific RNAi such as small interfering RNA, micro RNA or short hairpin RNA mediated gene silencing process. So, we can generate cancer lethality signatures for different cancer types and thus representing potential drug targets for recovery.\

## Conflict of Interest

Authors declare no conflict of interest.

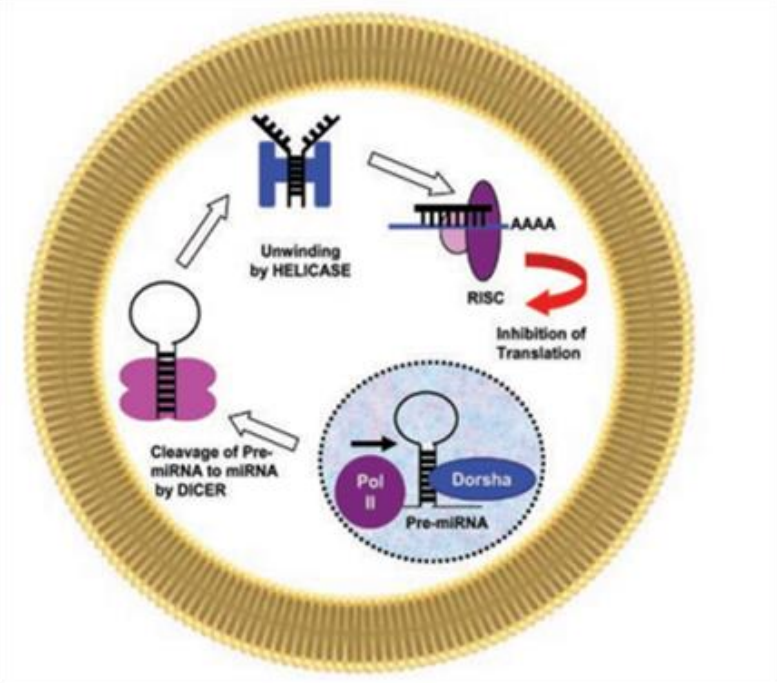


**Fig. 1:** Biogenesis of miRNA. The biogenesis of miRNA involves several enzymatic steps. Following transcription by RNA polymerase II (Pol II), capped and polyadenylated primary miRNA transcripts (primiRNAs) are processed in the nucleus by the endonuclease Drosha into one or more pre-miRNAs (1). This pre-miRNA is exported from the nucleus to the cytoplasm and processed by another RNase enzyme called Dicer, which produces a transient 19- to 24-nucleotide duplex (2). The duplex is cleaved (3), and only one strand of the miRNA duplex (mature miRNA) is incorporated into the RISC (RNA-induced silencing complex), which retains only the single-stranded mature miRNA (4). This miRNA-programmed RISC negatively regulates the stability and/or translation of target mRNAs, depending on the degree of complementary sites between the miRNA and its target

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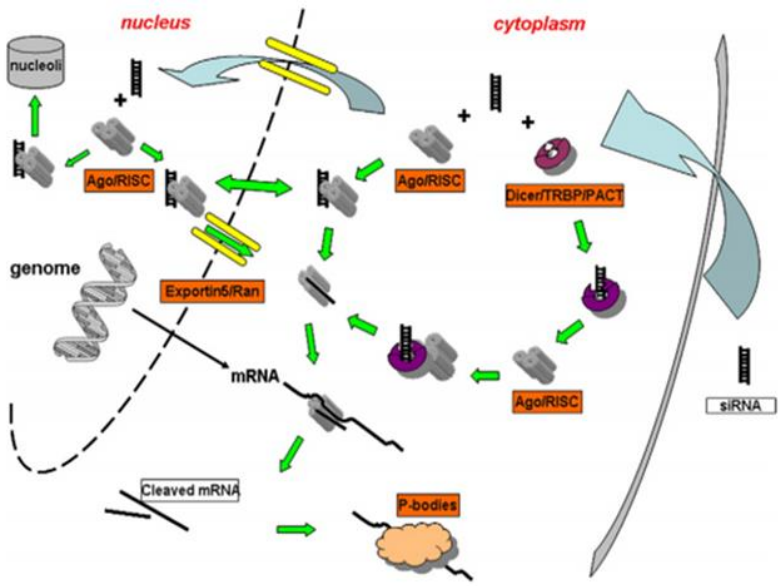


**Fig. 2:** Schematic of the siRNA mediated RNA interference pathway. After entry into the cytoplasm, siRNA is either loaded onto RISC directly or utilize a Dicermediated process. AfterRISC loading, the passenger strand departs, thereby commencing the RNA interference process via target mRNA cleavage and degradation. siRNA loadedRISCs are also found to beassociated with nucleolus region and maybe shuttled in and out of nucleus through an yet unidentified process

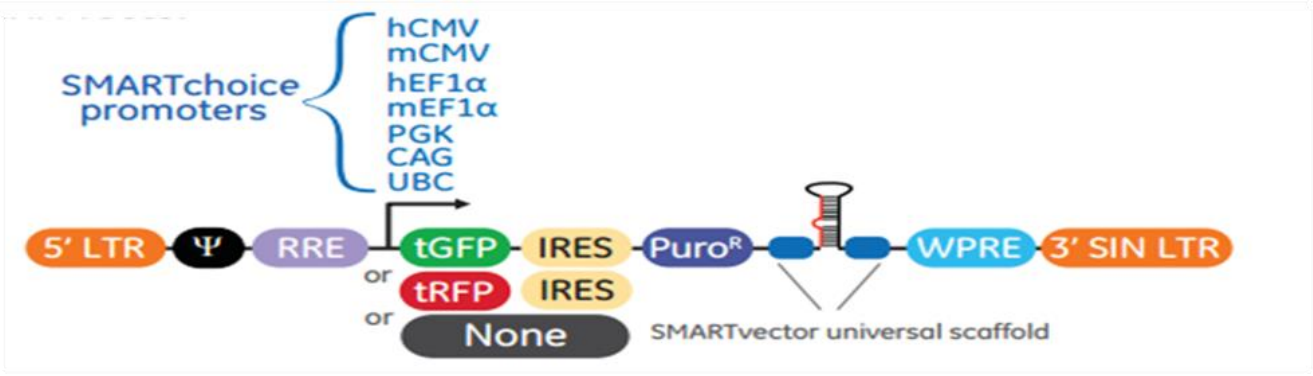


**Fig. 3:** Elements of the SMARTvector Lentiviral shRNA vector

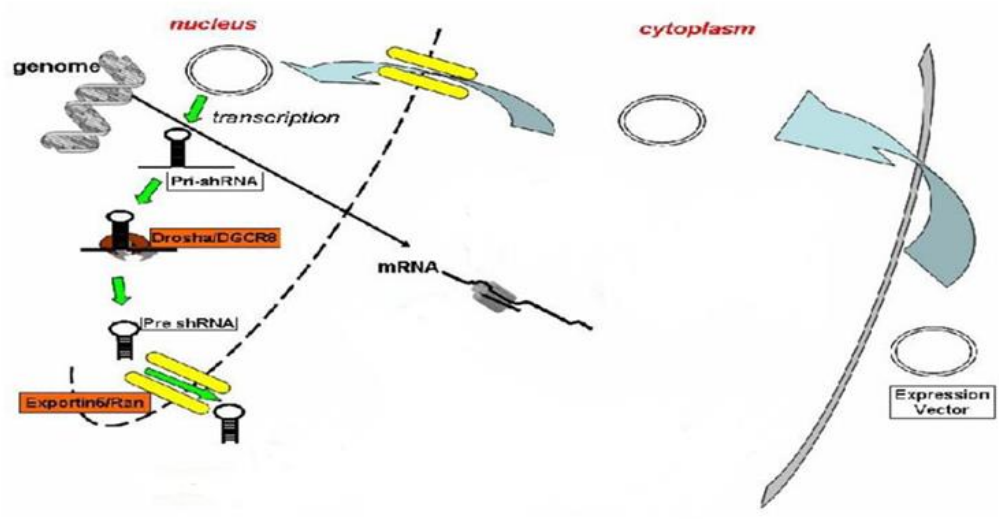


**Fig.** 4: Schematic of the shRNA mediated RNA interference pathway. After delivery of the shRNA expression vector into the cytoplasm, the vector needs to be transported into the nucleus for transcription. The primary transcripts (pre-shRNA) follow a similar route as discovered for the primary transcripts of microRNA. The primary transcripts are processed by the Drosha/DGCR8 complex and form pre shRNAs. Pre-shRNAs are transported to the cytoplasm via exportin 5

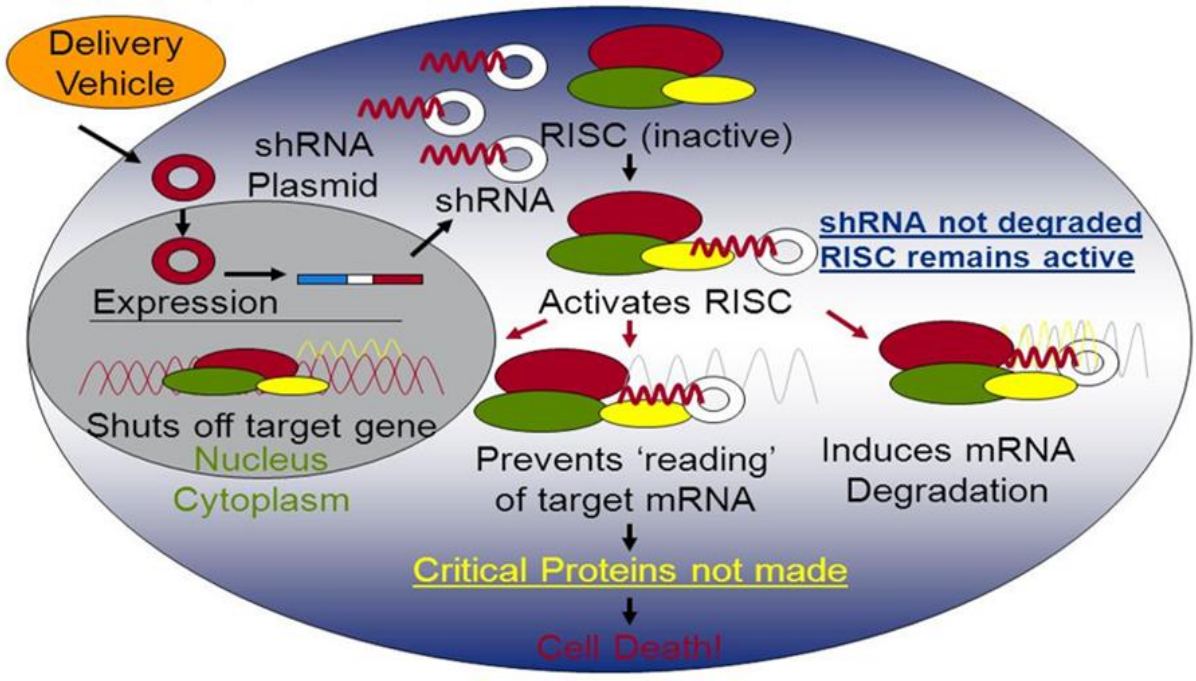


**Fig. 5**, When shRNA binds to the RISC complex then it's activates and function in three way. First shuts of target gene in to the nucleus. Second prevents reading of target mRNA in the cytoplasm. Third induce mRNA degradation in the cytoplasm

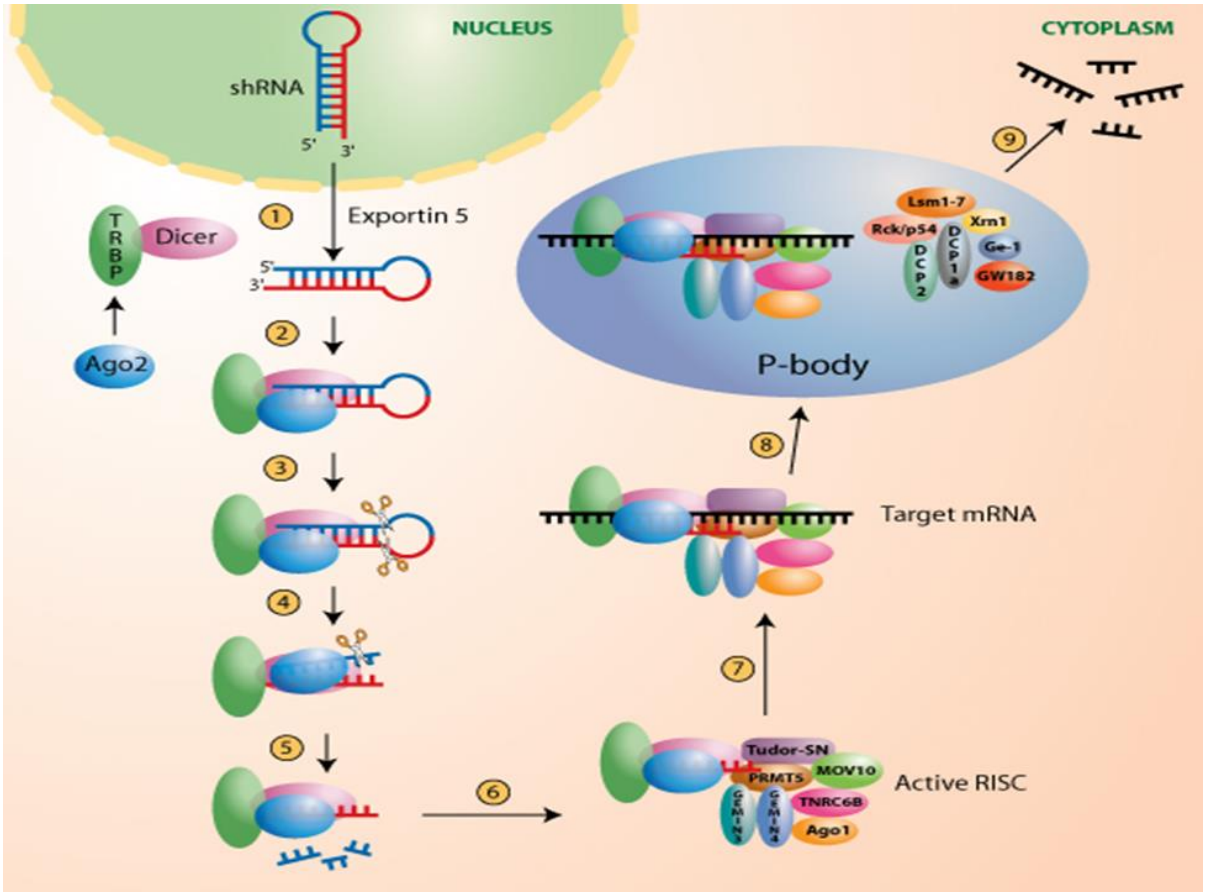


**Fig. 6:** Gene silencing in mammals

(1) Plasmidexpressed short hairpin RNA (shRNA) requires the activity of endogenous Exportin 5 for nuclear export. (2) Ago2 (Argonaute 2) is recruited by TRBP [Chendrimada TP. et al., 2005], that forms a dimer with Dicer and then receives the shRNA. (3) The shRNA is cleaved in one step by Dicer generating a 1923 nt duplex siRNA with 2 nt 3' overhangs. (4) After identification of the "guide strand" in the siRNA duplex, the"passenger strand"is cleaved by Ago2. (5) The "passenger strand" is released. (6) The"guide strand"is integrated in the active RNA Interference Specificity Complex (RISC) that contains different argonautes and argonauteassociated proteins. (7) The siRNA guides RISC to the target mRNA. (8) RISC delivers the mRNA to cytoplasmic foci named processing bodies (Pbodies or GWbodies) where in mRNA decay factors are concentrated. (9) The target mRNA is cleaved by Ago2 and degraded

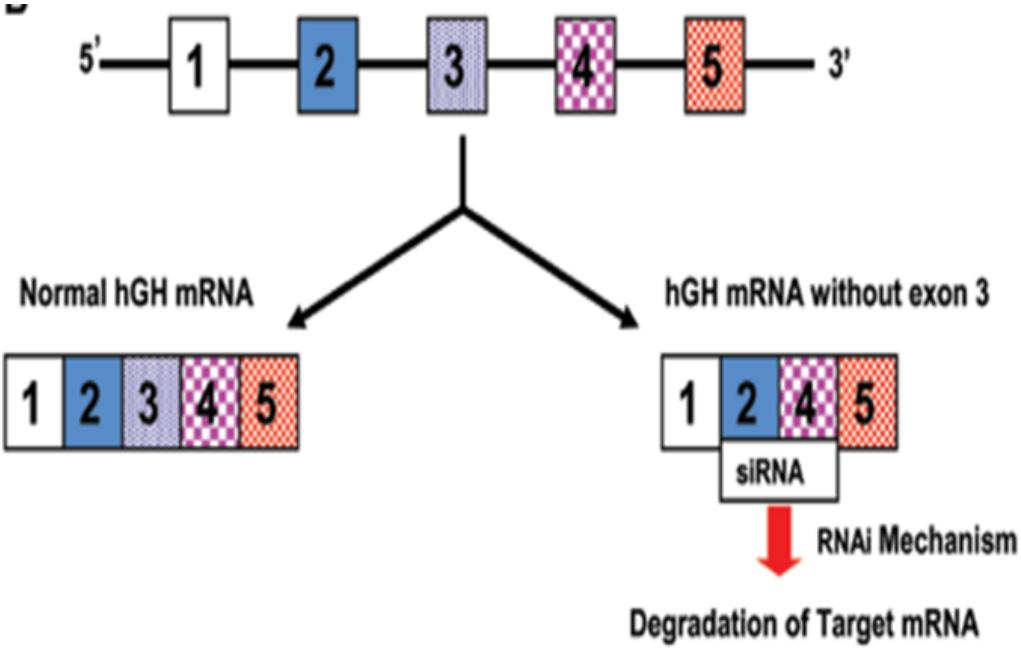


**Fig. 8:** RNAi-mediated silencing of human growth hormone (hGH) splice variant without exon 3

**Table 1**: Function of several components of SMART vector

| Vector Element | Utility |
|---|---|
| 5' LTR | Necessary for lentiviral particle production and integration into host cell genome |
| Ψ | Viral genome packaging sequence using lentiviral packaging systems |
| RRE | Enhances titer by increasing packaging efficiency of full-length viral genomes |
| tGFP or tRFP | TurboGFP or RFP reporter for visual tracking of transduction and expression |
| None | No reporter option for use in applications where fluorescence is not required |
| Puro $^{R}$ | Permits antibiotic-selective pressure and propagation of stable integrants |
| Scaffold | microRNA-adapted shRNA for gene knockdown |
| WPRE | Enhances transgene expression in the target cells |
| 3' SIN LTR | 3' Self-inactivating Long Terminal Repeat for increased lentivirus safety |

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