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RESEARCH ARTICLE

RNAI TECHNOLOGY AND ITS APPLICATION-REVIEW

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Abstract

RNA interference (RNAi), an evolutionarily conserved mechanism triggered by double-stranded RNA (dsRNA), causes gene silencing and inhibit translation in a sequence-specific manner. This technique was first time discovered in Nematode Caenorhabditiselegans.The specificity and robustness of RNAi have triggered an immense interest in using RNAi as a tool in various settings which results intovarious significantachievements in the area of therapeutics, crop protection, crop production, weed management etc. Furthermore, it has found to have immense potential in controlling agricultural and non agricultural pest such as pathogen i.ebacteria, fungi & virus, insect pest and nematodes. Moreover, RNAi leads to theenhancement of micronutrient or macronutrient bioavailability, improved salinity and droughttolerance as well as enhanced resistance toextreme hot and cold temperature tolerance.In this paper, we first reviewed the RNAi technology and its mechanism and then focused on of its applications in agriculture and biomedical research such as treatment for HIV, viral hepatitis and several other diseases.

Introduction:-

The mechanism of RNAi mediated gene silencing was first time studied by Mello and Fire in 1998 in Nematode.RNAi is also known by other nameas post transcriptional gene silencing.It is a phenomenon that generally occur inside a cell which targetedly select mRNA strand and chop them up. It is a whole process inside eukaryotic cell or prokaryotic celland various evidences of RNAi were observed in bacteria, virus,fungi,algae and insects, as well as plants and animals. It is very much expedient in plant biology as RNAi select a target i.e sequential selection of particular target and then breakdown that target . ConsequentlyRNAi target a common protein coding RNA which is also known as mRNA and then break it down so that there will be no protein synthesized from the mRNA. Generally RNA is a single stranded if by any chance they produce dsRNA which can be very dangerous because that dsRNA can be very specific for target site to produce RNAi and to ultimately collapse the actual protein coding RNA or mRNA because fundamental law of producing miRNA (micro RNA), shRNA(short hairpin RNA) and siRNA(small interfering RNA) are the dsRNA inside the cell and these RNAi produced can lead to silencing of the mRNA known as RNA mediated gene silencing and they bring out this gene silencing process by combining many other protein together and that protein complex is called as RISC.This RISC factor with this siRNA, miRNA or shRNA ultimately silence themRNA or degrade that RNA into smaller fragments as a result of which protein synthesis will not be possible. To produceRISC they require certain enzyme molecule to

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be associated such as slicer and Argonaut protein. But all above there is a another protein known as DICER which is RNase III type of an enzyme which is double stranded RNA specific endonuclease and which breakdown the RNAfrom the middle and produce siRNA, miRNA or shRNA. Hence, this RNAi mediated gene silencing has lead to profound achievement or success in therapeutics for treatment of cancer and other human and animal diseases. RNAi based technology have shown potential in agricultural and non agricultural plant crop. Furthermore, this post transcriptional gene silencing techniques shown a positive results in controlling plant infection or disease such as bacterial disease , viral disease, fungal disease etc whereas it is successful in managing insect pest of crop and even successful expressing gene in biological control agent for enhancing tolerance for extreme heat or cold. Moreover, RNAi leads to Enhancement of micronutrient or macronutrient bioavailability, Improved Salinity and Drought tolerance and enhanced resistance to extreme hot and cold temperature tolerance. Hence, the experimental study and results from RNAi based gene silencing sounds amazing. Altogether, serious consideration and acceptance and experimental approach should be encouraged.

Applications of RNAi in various areas

RNAi and its role in stress tolerance

Stress is usually defined as an external factor that exerts a disadvantageous effect or harmful effect on the plant. Abiotic stress causes the serious damages for the life on the earth particularly to the plant by negatively affecting its growth and yield. It has been estimated that nearly 70% of crop yield is reduced due to the abiotic stress. Now days, RNAi technology has been evolved a modern approach for gene function analysis and in translational research Programme. Recent findings of Donging yang et al., 2020 demonstrated an experiment on sweet potato against drought stress tolerance where they found that IbINH level increases in response to stimuli like droughts, jasmonic acid treatment ,salicyclic acid and abscisic acid (ABA).Furthermore, two transgenic line of sweet potato were developed i.e SI and RI.Where excessive upregulation of IbINH in sweet potato(SI) resulted into decrease in plant growth and increase in drought tolerance and lowering the expressing of IbINH in sweet potato (RI) by RNAi technology which resulted into vigorous growth and drought sensitivity manifest the RNAi is playing an important role in abiotic stress stimulation in different crops. RNAi technology may be a substitute of complex molecular techniques because of containing several benefits, its specificity and sequence based gene silencing. Due to this property, RNAi has been effectively utilized for incorporating desired trait for abiotic stress tolerance in various plant species

RNAi as a potential therapeutic for humans; genetic diseases

Dominant negative genetic disorders, in which a mutant allele of a gene causes disease in the presence of a second, normal copy, have been challenging since there is no cure and treatments are only to alleviate the symptoms. Current therapies involving pharmacological and biological drugs are not suitable to target mutant genes selectively due to structural indifference of the normal variant of their targets from the disease-causing mutant ones (Table 1). In instances Although there is a cooling trend by the pharmaceutical industry for the potential of RNA interference (RNAi), RNAi and other RNA targeting drugs (antisense, ribozyme, etc.) still hold their promise as the only drugs that provide an opportunity to target genes with SNP mutations found in dominant negative disorders, genes specific to pathogenic tumor cells, and genes that are critical for mediating the pathology of various other diseases (Seyhan, 2011).

A promising lead toward using RNAi for the treatment of genetic diseases has been provided by preliminary studies demonstrating how single nucleotide polymorphisms (SNPs) in mutant allele transcripts can be used as selective targets for RNAi. Disease causing polyglutamine proteins encoded by CAG repeat containing transcripts found in several neurological diseases present especially challenging targets because CAG repeats are common to many normal transcripts as well, and cannot be selectively targeted by siRNAs. Alternatively, single nucleotide polymorphisms are very often found in mutant allele transcripts, and represent potential selective targets. Systematic analyses of siRNAs in which the polymorphic nucleotide is complementary to the mid region of the siRNA provides an siRNA/SNP combination that is highly selective. In certain examples, the siRNAs direct selective degradation of only the mutant transcripts, leaving the wild type transcripts intact despite having only a single mismatch with the wild type sequence (Miller et al., 2004a, Miller et al., 2004b). Particular purine–purine mismatches at positions 10 and 16 relative to 5′ end of the guide strand provide selectivity (Schwarz et al., 2008). Since the wild-type SOD1 performs important functions it is important to selectively eliminate the expression of only the mutant allelic transcript. Many SOD1 mutations are single nucleotide changes. Since delivery of siRNAs and viral vectors expressing siRNAs to affected regions of the brain is technically feasible (McCaffrey et al., 2003), the promise of clinical use of RNAi for treatment of degenerative, neurological diseases may approach reality soon. Despite the

excitement and promise of therapeutic RNAi, there are many obstacles, the greatest of which is delivery. Systemically delivered siRNAs face degradation by nucleases, and the use of viral vectors to target organs of interest is still in its infancy.

RNAi in gene regulation and antiviral responses

RNAi and RNAi-related mechanisms play essential roles in the regulation of cellular gene expression, as well as in innate antiviral immune responses. RNAi is regarded as a natural defense mechanism against mobile endogenous transposons and invasion by exogenous viruses which have dsRNA as an intermediate product. With this defense mechanism, organisms maintain genetic integrity and hinder infection (Ebbesen et al., 2008). For many applications, it may be complicated to introduce short dsRNAs directly into cells. However, many groups have now shown that appropriately designed DNA molecules containing inverted repeat sequences can be transcribed into RNA molecules that form RNA hairpins. If the sequences are chosen correctly, these are processed by the Dicer nuclease to form siRNAs. Thus all the methods derived for delivering genes into cells can in principle be used to deliver siRNAs as well. This made the application of RNAi therapy for the prevention and treatment of viral infection convenient RNAi has got a potential for the treatment of viral diseases such as those caused by the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV). However, there are important issues and concerns about the therapeutic application of this technology, including difficulties with delivery and uncertainty about potential toxicity that needs to be solved. The HCV genome is a positive-strand RNA molecule with a single open reading frame encoding a polyprotein that is processed post-translationally to produce at least ten proteins. The only therapy currently available uses combined interferon (IFN) and ribavirin. Subgenomic and full-length HCV replicons that replicate and express HCV proteins in stably transfected human hepatoma cell-derived Huh-7 cells have been used to study viral replication and the effects of various antiviral drugs. Small inhibitory RNAs targeting the internal ribosome entry site (IRES) and non-structural protein NS3 and NS5b encoding mRNAs were shown to inhibit HCV replicon function in cell culture (Wilson et al., 2003). Furthermore, anti-HCV siRNAs were shown to “cure” Huh-7.5 cells bearing persistently replicating HCV replicons. Delivery of the siRNAs or vectors that carry siRNA expression cassettes is the major challenge for treatment of HCV. The method of delivery used in a number of in vivo studies, hydrodynamic intravenous injection, is not feasible for the treatment of human hepatitis. Delivery is a problem that must be confronted for any therapeutic application of RNAi. A recent report demonstrates that it is feasible to introduce genetic material into hepatocytes using catheters or even localized hydrodynamic procedures (Eastman et al., 2002). Numerous studies have established the proof of concept that diseases can be targeted by therapeutic RNAi, and several small interfering RNAs (siRNAs) are currently being tested in clinical trials (Grimm and Kay, 2007). Despite these rapid advances, significant hurdles still need to be overcome for the widespread therapeutic application of siRNAs. Perhaps the greatest challenge is the delivery of effective quantities of siRNAs into the cytoplasm of relevant target cells in vivo (Dykxhoorn and Lieberman, 2006).

HIV was the first infectious agent targeted by RNAi perhaps owing to the fact that the life cycle of HIV is well understood as is its pattern of gene expression. Synthetic and expressed siRNAs have been used to target a number of early and late HIV-encoded RNAs including the TAR element, tat, rev, gag, env, vif, nef (Jacque et al., 2002) and reverse transcriptase. Cellular cofactors, such as NFκβ, the HIV receptor CD4 and co-receptors CXCR4 and CCR5 have also been successfully down regulated by RNAi resulting in an inhibition of HIV replication. Moreover, inhibition of HIV replication has been achieved in numerous human cell lines and primary cells including T lymphocytes and hematopoietic stem cell derived macrophages.Despite the success of in vitro RNAi-mediated inhibition of HIV-1, for future clinical applications, targeting the virus directly represents a substantial challenge since the high viral mutation rate will certainly lead to escape mutants (Boden et al., 2003). RNAi-mediated down regulation of cellular co-factors required for HIV infection is an attractive alternative or complementary approach.

Delivery of siRNAs or shRNA encoding genetic units to HIV infected cells is also a challenging problem. The target cells are primarily T lymphocytes, monocytes and macrophages. Since synthetic siRNAs will not persist for long periods in cells, delivery would have to be done repetitively for years to effectively treat the infection. Systemic delivery of siRNAs to T lymphocytes is a major barrier and probably not feasible. T-cell isolation from patients followed by transduction, expansion of the transduced cells and re-infusion has been found to be the preferred path (Dropulic, 2001; Davis et al., 2004).

There are additional challenges for using siRNAs in the treatment of HIV-1 infection, including validating the approach in a relevant animal model and preventing the emergence of variants resistant to treatment because of the high sequence diversity of the virus. In this issue, Kumar et al. (2008) exploit a series of recent technical advances to

overcome these obstacles. They demonstrate that targeting a combination of host and viral proteins with siRNAs can efficiently inhibit HIV-1 infection in a humanized mouse model. It has been shown previously that antibodies can be used to deliver siRNAs into the cytoplasm of specific target cells (Song et al., 2005). This approach decreases the amount of siRNA that is needed, thereby minimizing the risk of undesired effects in bystander cells. In their new work, Kumar et al. use a single-chain antibody to the CD7 receptor conjugated to a nonamer arginine peptide (9R) (Fig. 1). The CD7-specific antibody is well suited for siRNA delivery because CD7 is expressed by most T cells and is rapidly internalized. Moreover, this antibody has already been used in clinical studies to target toxins to T cell lymphomas and leukemias. In previous work, Kumar et al. (2007) demonstrated that the positively charged 9R peptide binds to polyanionic nucleic acids and can be used to deliver siRNA to neuronal cells. They now show that these techniques can be used to suppress HIV-1 replication and prevent CD4+ T cell depletion in vivo in a humanized mouse model of acquired immune deficiency syndrome (AIDS). This is a significant advance, not only because these findings enhance the prospect of a new HIV-1/AIDS therapy but also because this study introduces an siRNA delivery system that could be adapted to target different receptors and hence other cell types. Moreover, given that the binding of the siRNA to the 9R tag is noncovalent, this approach should make it possible to easily compare the efficacy of different siRNAs

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