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Annals of Biological Research, 2012, 3 (11):5119-5126
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Gene Regulation Control by RNA

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ABSTRACT

Evolutionary conserved sequence specific gene regulation mechanism "RNAi" is a powerful tool for understanding the molecular pathways, genetic screening of desired genes and silencing the targeted genes. Although, RNAi mechanism is a universal system then present both in many eukaryotes and also prokaryotes with similar complicated mechanism, proteins that involved in the mechanism are shows minor differences. Hence the diversity of the proteins within the mechanism is also allowed to scientist to find phylogenetic relationship within the organisms. But the major importance of the mechanism is comes from its agricultural and pharmacological potential. RNAi guided solutions such as value-added products or patient specific new therapeutics by rational design of the molecular pathways via RNAi mediated gene silencing.

Key Words: RNAi, Gene Silencing.

INTRODUCTION

Post-transcriptional gene silencing (PTGS), were first recognized as unexpected perfect regulatory system in limited group of organism. Nowadays, universal gene-regulation system is crucial both understanding gene function and control structural, enzymatic and regulatory processes. RNA silencing is a regulatory system within living cells that controls gene expression in post transcriptional or in some cases [RNA-directed DNA or histone methylation] in transcriptional manner [1]. That system is evolutionary conserved process that mediates sequence-specific mRNA degradation among plants, fungi, insects and animals. In addition that this system known as RNA interference in animals, co-suppression in plants, RNA quelling in fungi [2] That conserved mechanism has been discovered in *Plasmodium falciparum* [3], *Trypanosoma brucei* [4], Planaria [5], *Arabidopsis thaliana*m [6], *Neurospora crassa* [7] *Caenorhabditis elegans* [8], *Drosophila melanogaster*[9], [10], [11], zebrafish [12], mice [13] to human [14] [15] [16]. RNA mediated gene-regulation system of endogenous or exogenous gene silencing is important function in biologic and metabolic process including genome maintanence [17], cell growth, differentiation [18], apoptosis [19], regulation of cell cycle, heterochromatin formation [20] [21].

For the first time, the RNAi mechanism was discovered in 1990 by Napoli and Jorgensen with their studies which covered over expression of chalcone synthase (CHS) in petunias. As a result of experiment, unexpected 50-fold lower expression of endogenous CHS transcript with phenotypic changes led them to hypothesize that introduced transgene cause co suppressing of homolog gene [22]. In 1992, similar RNAi mechanism was recognized *in Neurospora crassa* that introduction of exogenous sequences cause repression of endogenous al-1 or al-3 genes and termed as "quelling" [23]. *Caenorhabditis elegans* one of the most important model organism in developmental genetics due to this advantage RNAi mechanism tested in *C. elegans* embryos for finding the effect of RNA injection on cell division direction. The aim of this experiment was to show the importance of par-1 in asymmetric cell divisions at *C. elegans* embryos by both sense and antisense par-1 RNA sequence injection [24]. Fire and Mello was performed an experiment to understand structure and delivery of the interfering RNA within the cells and obtained an surprising result that showed double-stranded RNA was more effective to produce interference response

then single strand silencing [8] RNA dublex (dsRNA) induce RNA interference response in cultured mammalian cells was first showed by Elbashir et al. in 2001. This discovery about antisense approach allow reverse genetic applications, by that way scientist understand disease related basic molecular mechanism and principles that regulate the post transcriptional gene silencing mechanism in mammalian cells. In 2001; RNAi has been awarded as "The Molecule of the Year" by the journal *Science* due to the importance of implementation of the mechanism. The popularity and applicability of the mechanism led to awarded Fire and Mello with Nobel Prize in Physiology or Medicine by their discovery about gene silencing mechanism in *Caenorhabditis elegans* in 2006.

Gene Regulation in Plants;

In plants, natural gene silencing mechanism "miRNA" is important tool to control cellular, physiological and developmental process. Aberrant expressions of miRNA genes provide to knock down target gene and help to understand metabolic pathways and environmental responses.

The experiments shows that RNA interference mechanism is an important regulatory tool for auxin signaling, organ separation and polarity, leaf growth, developmental transitions, floral organ identity and reproductive development in plants [25]. Currently, the most excited process of sequence specific gene silencing is to gain viral resistance against RNA or DNA viruses in the field crops because of their economic importance. Plant viral diseases cause severe damage and economic losses in vegetable and fruit crops by reducing vigor, yield and product quality [26]. In fact, RNAi are ancestral mechanisms that provide maintaining the integrity of the genome against transposable elements and viruses. Virus induced gene silencing has an enormous power to down regulate the virus specific endogenous genes [27]. Thus mimicking the natural mechanism able to mediate viral response and inhibit agricultural loss in valuable crop species [28].

The major point in mediating viral response is to activate mutable virus response, since crops are infected with more than one virus in natural conditions [29]. Jan and colleagues were activated mutable viral resistance by single chimeric transgene that contains N gene segments of tomato spotted wilt virus (TSWV) and CP gene of turnip mosaic virus (TuMV). That fusion protein consists of two different fused gene segments of different viruses linked to a `silencer' DNA and trigger RNA-mediated virus resistance by transformation [29]. RNAi mediated viral resistance mechanism able to apply against DNA viruses; yet Bian was showed efficiency of the mechanism is lower than RNA viruses [30] [31]. However, a recent study about virus resistance via gene silencing was showed that mechanism is an important tool to protect the crops against emerging DNA viruses like geminivirus and RNA viruses that contains linear or fragmented genome [27].

In plant biotechnology applications, RNAi has crucial value to improve of plant productivity and nutritional value [32]. The first application of antisense technology in plants was obtained by reduction of polygalacturonase activity in tomato fruit. In fact the importance of antisense technology comes from its power that allows researchers to both interfered and understand the metabolic pathways by metabolic engineering. The metabolic engineering approach provides to improve desired cellular properties by rational genetic modifications including RNA interference. RNAi technique is important approach to down regulate or up regulate desired key player, thus RNAi designed and employed to leans on direct perturbations of the metabolic network to improve desired property. One of the good examples with possible large-scale commercial use is producing caffeine-free tea or coffee. To produce caffeine free tea, complementary antisense molecules triggers targeted caffeine genes by that way inhibits target protein synthesis on transcript level [33]. Toxic gossypol elimination from cotton seed was achieved by reducing Delta-cadinene synthase gene expression by RNAi mechanism during seed development [34]. Metabolic engineering by RNA silencing is also useful method producing value added products such as plants with enhancing caroteinoid and flavonoid content, improving fatty acid composition, increasing essential amino acids and protein quality. That approach also applicable for producing commercially important products in example reduced lignin content in alfa alfa plant for better feed and industrial applications. Reducing lignin content has economical and environmental benefits because less chemicals are used for delignification [35].

Gene Regulation in Medicine;

The development of RNA interference mediated gene silencing, to down regulation of abnormally or constitutively expressed molecular targets especially in cancer, infectious, autoimmune and neurodegenerative diseases are promising approach to improve the efficacy treatment for personalized therapy. RNAi based therapeutics are designed behind the fact that miRNA expression profiles are changed in target tissue versus healthy tissue. Thus exploring differentiate miRNA profile, understanding disease related miRNA dysregulation and containability of the target genes are critical steps to developed RNAi based therapeutics.

A large number of infection relevant process have been subjected to RNAi, that caused by human immunodeficiency virus type-I (HIV-I), hepatitis virus and influenza virus. Many therapeutic approaches failed to success due to high

mutation rate and complex pathogenesis of viruses especially HIV [36]. Inhibition of HIV infection by RNAi pathway can be performed by silencing the CD4 surface expression and reduced CD4 receptor amount in surface [37], [38] or down regulation of co-receptors such as CXCR4, CCR3 and CCR5 [39] [40]. The other way is also inhibition of HIV encoded major regulatory genes related to HIV infection like p24/Gag, Nef, Vif, Ref, Rev LTR, [41], [42] [43] [44] to reduce virus production. However, human immunodeficiency virus capable to develop resistance against RNAi based antiviral agents due to its high mutation rate. In this circumstance, introducing different siRNAs targets that directed another zone of the same mRNA, is one of the best solutions to over-come that resistance problem [45]. Thus, innovation of shRNA based drugs that efficiently target HIV-1 escape routes; help to both over-come evolution pressure and lead innovation of new drugs aside understanding the HIV evolution mechanism [46].

Persistent Hepatitis B and C virus infection are also requires novel antiviral agents and therapeutic strategies since treatment options for chronically infected patients are limited [47], [48]. In that point, novel therapeutic approaches are required to effective treatment. RNAi based therapeutics are promising new treatment approaches for both hepatitis B and C virus infection [49] [50] [51]. Hepatitis C virus is affecting the liver and cause chronic infection with its single positive-stranded viral RNA. RNAi based therapeutic approach for HCV infection is comparatively easy behinds these facts: first, viral vectors has capable to infect liver by natural ways, second ability of efficient delivery to hepatocytes and third lack of ability to create re-infection by degradation of RNA molecules [52]. Liver specific “miR-122” is responsible to regulate hepatitis C virus translation, by that way cause chronic hepatitis C infection [53] [54]. Due to crucial role of mir-122 in viral infection and advantage of easily targe to liver, Mir-122 based therapeutics was produced by Santaris Pharma and in its phase I clinical trial stage for HCV infection [55].

Cancer is a genetically based multi factorial disease that occurs with different reasons which includes proto-oncogene activation, oncogene cooperation, predominant point mutations and loss of tumor suppression activities. As a result of that reasons, breakdown of the regulatory mechanism and abnormal proliferation of normal cells cause cancer cells escape from normal cell behavior and shows unlimited growth. Eventually, consistent cell division accompany invasion of the neoplastic cells among normal tissue and organs. The main problems, hundred distinct types of reason triggers neoplasm in cellular level thus, no common protocol to reduce a theory into standard therapy for cancer. The most effective way for cancer treatment is firstly, understanding the specific molecular abnormalities that lead up to cancer, then rational drug design that targeted against specific cancerous tissue. Recent research’s proved that miRNAs expression among cancer tissues differs then corresponding noncancerous tissue, thus discovering the novel mediators, targets and pathways are provide a broad overview of understanding. MicroRNA expression profile shows dramatic changes in various cancer type by effecting the gene expression of miRNA-target tumor suppressors or oncoproteins [56], [47], [57], [58], [59], [60]. miRNA upregulation or down regulation results tumor suppression or oncogene activation within cancer cells [61], [62], [63]. Experimental data suggested that some miRNA has oncogenic function as mir17-92 (64), [65] and mir-31 [66] in lung cancer, miR-155 in breast cancer [67], mir15&mir16 in CLL and prostate cancer [68], [69]. On the other hand, some other miRNA molecules act as tumor suppressor, especially let-7 [70], [71], [72], mir-128 [73], [74], miR-218 [61] and p53 regulated mir-34 family [75], [76]. RNAi based therapeutics able to acts in cancer therapy with two different way: (1) Silenced the over-expressed endogenous miRNAs [OncomiRs] which initiate cancer development by chemically engineered oligonucleotides which called as ‘antagomirs’ (2) Enhaced the lower expressed endogenous miRNAs (TSmiRs) which prevent tumor development. Therefore cancer cells can be destroyed and eliminated without damaging normal cells and by that way minimize adverse side effects. As a sum, commercial RNAi based systems is a useful tool for control and regulation of cell cycle, stimulation of apoptosis and angiogenesis, down regulation of over-expressed oncogenes, inhibition of metastatic growth and migration, enhancement efficiency or reduce side effect of traditional chemotherapy and radiotherapy [77], [78], [79], [80], [81].

Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, and Amyotrophic lateral sclerosis (ALS) are common age related disease results from neurodegeneration or dysfunction of specific neurons. Many neurodegenerative diseases are based on gain-of-function mutations; hence disease specific genetic alterations result accumulation of disease related toxic compounds, thus these diseases able to prevent by RNAi based pharmaceuticals. For example, Spinocerebellar ataxia is a progressive disorder of brain function and elevated level of human ataxin-1 expression is characteristic for Spinocerebellar ataxia type 1 (SAC1). According to Harper’s research which performed in animal model characteristic ataxin-1 inclusions in Purkinje cells able to resolve by shRNA molecules directed against human ataxin-1 [82]. The other well known neurodegenerative disorder “Huntington’s disease” result from gain of function mutation on the protein huntingtin (htt) [82], [83], , and aggregation of mutant Huntingtin protein (mHtt) cause neurodegenaration. The experiments which performed in cell culture and mouse model indicates targeted htt gene expression by RNAi mechanism reduce disease symptoms [82], [84]. Three-quarters of the United States and European Huntingtin Disease patient populations share same single-nucleotide polymorphism (SNP) in HD. Thus strategies for knocking down toxic Huntington protein by anti-human

huntingtin siRNA is promising novel therapy for cure and improving life quality [83], [85]. Nowadays, allele selective inhibition of mHtt expression is aimed to reduce toxicity and enhances selectivity [85].

Metabolic disease is an umbrella term that associated with many burdening comorbidities, such as obesity, diabetes, high blood pressure and high cholesterol. Nowadays, the researchs aim to diagnose that metabolic disease associated comorbidities due to targeting common pathways by RNAi based therapeutics [86]. Diabetes is a good example for RNAi mediated gene therapy for autoimmunity suppression or regulation of insulin levels and hyperglycemia which correlated with tissue and vascular damage. Novel RNAi based therapeutics gave promising results to inhibit diabetes related genes like Nramp1 [87] [88]. Animal models showed that Protein tyrosine phosphatase 1B [PTP1B] able to be major therapeutic target for the treatment of type II diabetes by siRNA expression vector, and its validated target in diabetes [89], [90]. Hypercholesterolemia is important metabolic failure for cardiovascular diseases and characterized by high levels of cholesterol in the blood. Hypercholesterolemia occurs due to environmental and genetic factors, and also in collaboration with other metabolic disease or syndromes such as diabetes mellitus type 2, hypothyroidism or nephrotic syndrome. Hypercholesterolemia is treated by medications or low- dietary cholesterol intake to reduce cholesterol level. Treatment of hypercholesterolemia with siRNA mediated knockdown of ApoB or PCSK genes are new approaches to aim decrease total cholesterol and low density lipoproteins (LDL) levels reduced at least 15% [55], [91].

CONCLUSION

RNA interference is new toys of all scientist for their innovation beyond their dreams, thus different application ranging from medical applications to biotechnology area is successfully achieved. The leader biotechnology companies from different area are applied for patent applications for their RNAi guided solutions.

In example, knocking down of genes are valuable laboratory tools to understand biological functions, evaluation molecular mechanism of metabolic pathways. Several appropriate tools induce RNA interference mechanism allows to regulate or *knock* silence specific genes in post transcriptional manner in desired developed strategies or tissue.

Plant biotechnology applications including improvement of plant productivity, quality and yield; producing value added crops, enhancement commercially important products, obtaining resistant plants are major economic advantage of reverse genetic applications.

RNAi applications over drug therapies are exhilarating step and new opportunities to treat incurable disease such as neurodegenerative diseases, Cancer and AIDS. The clinical success of RNAi based therapeutics comes from its advantage over traditional pharmacologic products like lower toxicity, enhanced pharmacodynamic and pharmacokinetics properties. On the other hand still lots of unknown immune related mechanism dismay the scientist. But, RNA based drugs provide greater efficacy in disease control and intervention, thus has bright future in the research and therapy of incurable disease. Immune system activation through Toll-like Receptors, unknown side effects or RNAi delivery efficiency are still main problem, so new strategies are required to overcome the limitations.

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