YOUNG SCIENTISTS journal

Gene silencing as a therapy for cancer

December 23, 2015 · Biology

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Nandakumar G. Gene silencing as a therapy for cancer. Young Scientists Journal. 2015 Dec 23 [last modified: 2015 Dec 23]. Edition 1.

Abstract

In this article I will explain what RNAi is, the genetic mechanisms behind it and why it deserves the considerable attention it has been receiving. Expanding on these basic mechanisms, I will go onto explore some of the latest RNAi trials involving Huntington's disease and cancer. I will highlight the complications associated with the treatment of these complex diseases and importantly, why RNAi is seen as a viable therapeutic pathway.

Introduction

Given that the expenditure for cancer care in the US alone is expected to reach $170 billion and that more than a third of men and women will be diagnosed with cancer during their lives[1] , there is an urgent need for effective treatments. Among the promising fields, RNA Interference (RNAi) and its role in gene silencing has drawn attention and seen steady progress over the years. Whilst the ability to turn genes on and off at will may seem like a biomedical superpower, the research brings this ideal closer to reality and application as a treatment.

What is RNAi?

RNAi is the process of gene expression silencing that occurs after transcription (the copying of information from DNA to RNA) which functions as a defence mechanism against viruses and transposable elements. Whilst this is a natural process allowing both a host and a parasite to manipulate one another's gene expression[4], it may also hold the therapeutic potential to silence causative genes involved in tumorigenesis[6].

RNAi was first documented in 1998 by Andrew Fire and Craig Mello who were investigating how gene expression is regulated in C. elegans, a nematode. They were injecting mRNA that codes for muscle protein production which, if incompletely expressed, results in twitching movements. When they injected the coding (sense) strand and the complementary template (antisense) strand separately they observed no twitching. When injected together however, they observed the twitching characteristic of dysfunctional muscle protein genes. Fire and Mello hypothesised that the double-stranded RNA formed by the binding of the sense and antisense strand was somehow silencing the gene carrying that same code[2]. This discovery and hypothesis, proven and recorded extensively, earned them the Nobel Prize in 2006 and has since become an area of extensive research.

Mechanisms of RNAi Gene Silencing

The process of RNAi is mediated by small interfering RNA,abbreviated to siRNA. It is worth noting that since the siRNA are double-stranded, the principle of the 'sense' and the 'antisense' strand also applies. The sense strands have a base sequence identical to that of the transcribed messenger RNA and the antisense strand has the complementary sequence[11]. SiRNA is produced in two stages, the starting and effecting stages. In the starting stage, long double-stranded RNA (derived from the gene which is to be silenced) is cleaved into short interfering RNA (siRNA) by an enzyme named Dicer. In the effecting stage, the two strands of siRNA are then separated and the antisense strand is transported to a group of proteins known as the RNA-induced silencing complex (RISC). The RISC uses the antisense strand (complementary to the target mRNA) and the ribonuclease activity of Argonaute to bind to and degrade the corresponding mRNA[3].

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Dicer, the ribonuclease that cleaves double stranded RNA into siRNA

Fig. 1: Dicer from G. intestinalis

Dicer, the ribonuclease that cleaves double stranded RNA into siRNA

Unfortunately, siRNA is known to have a few drawbacks in practice; importantly, 99% of siRNA is degraded after 46 hours[5] making stable gene silencing unobtainable. Furthermore, there is a high rate of off-target effects including inflammation, cytotoxicity and stimulation of the immune system[8].

Another method of post-transcriptional silencing was then developed utilising short hairpin RNA (shRNA), improving upon many of the disadvantages of siRNA. Unlike siRNA, synthetic shRNA continues to be synthesised in the nucleus via elements of the inbuilt RNAi machinery allowing for more stable gene knockdown in cell lines. ShRNA must be introduced using an expression vector; a plasmid designed for protein expression in cells. The vector introduces the desired gene into the cell where the mechanisms for protein synthesis are 'borrowed' producing the protein encoded by the gene, in our case, pre-shRNA. The long pre-shRNA is then cleaved into much smaller hairpin shaped RNA, shRNA, by Dicer. Once again, the antisense strand is introduced to the RISC where it can bind to and degrade the target mRNA[7].

An schematic illustrating the mechanism of shRNA action.

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Fig. 2: shRNA plasmid gene silencer

An schematic illustrating the mechanism of shRNA action.

Although shRNA requires a dose of only 5 copies[5] and is expressed up to 3 years after introduction, complications arise in vivo as mammalian cells contain a number of endogenous systems known to complicate the application of shRNA. Chiefly, the protein kinase PKR suppresses duplexes longer than 30 nucleotides[9], limiting the length, and by extension, the functionality of shRNA.

Notable siRNA/shRNA trials

Since RNAi relies on highly sequence-specific interactions between RNA, synthetic siRNA/shRNA can be tailored to silence nearly any gene encouraging the clinical trials of 21 different drugs targeting 14 diseases[10]. As expected, the trials are mixed as attempting to successfully manipulate a process so intricate uncovers new obstacles when functioning in parallel to a host of other cellular functions.

In Vitro

In vitro studies of chronic myeolid leukaemia have shown siRNA successfully knocking down the expression of BCR-ABL, a protein that prevents imatinib, a chemotherapy treatment for CML, binding to cancer cells. The results show lower levels of transformed haematopoietic cells spreading throughout the body indicating that the target cells were much more vulnerable to imatinib[12]. Further in vitro studies involving the knockdown in expression of antiapoptotic proteins clustering and surviving in cancer cells have proven successful, leaving those cells more vulnerable to chemotherapy treatments[13].

In Vivo

In vivo treatments present the additional challenge of effective delivery; the improvements in commercial transfection agents has aided siRNA growth but the use of adenoviruses and lentiviruses for shRNA delivery harbours well-known toxic effects. Attempts have however been made to overcome this obstacle by inserting shRNA 'cassettes' into different sites within the vector's genome, altering the vector's genome itself and examining the effect this has on shRNA expression. It has only recently been found that adenovirus vectors express viral-associated RNAs that not only trigger severe immune responses but also inhibit RNAi pathways by saturating Dicer and RISC. The insertion of the shRNA into a different position and the use of VA-deleted adenovirus has been shown to reduce the drawbacks of this method and increase shRNA activity[14]. Whilst this approach has been applied to suppress hepatitis C replication[10], the opportunity to apply the method to cancer models still remains.

Another clinical trial that has drawn significant attention is being carried out by UCL and Isis pharmaceuticals and is focused,(in its early days), on safety as opposed to the complete knockdown of mutant Huntington expression. There are currently no treatments to prevent or even slow the progression of Huntington's but RNAi gene silencing is the first therapeutic pathway to be trialled[15]. Whilst the results have not yet been published, we can see that the nature of RNAi can make it a versatile therapeutic tool against a number of genetic disorders.

Ex Vivo

Ex vivo delivery of the therapeutic is also an option that has been applied by Duke University in a phase 1 study. The procedure, known as autologous cell therapy involves removing cells, modifying them with the appropriate treatment and re-implanting them into the patient. In a study targeting metastatic melanoma, the siRNA should enhance antigen presentation of the melanoma provoking a strong immune response, theoretically. Promisingly, according to Dr. Pruitt from the University, none of the 10 patients have experienced toxicity from the process and have all shown antigen-specific immune responses[10] .

Why is cancer so difficult to treat?

Having explored the potential of RNAi, it is reasonable to ask why cancer remains so difficult to treat. The simple explanation is that cancer is not one disease but many hundreds of diseases. Whilst they may share common themes such as a resistance to apoptosis, forming their own blood supply (angiogenesis) and the ability to metastasise, each cancer achieves these in distinct ways. Even cancers of the same cell type can have quite different characteristics and within each cancer there are different populations of cells[16]. As a result, finding a single cure for cancer, even aided by discoveries such as RNAi, is unlikely. Instead, there must be numerous approaches to the problem, and among these, RNAi proves especially promising. It can be tailored to desired genes, allowing for specialised drugs for specialised diseases.

Conclusions

The problem cancer presents lies in its specificity, making an umbrella treatment unlikely. Methods such as RNAi, however, provide an alternative approach that may not alone provide an effective treatment by itself but as we have learned, can work well in parallel with current chemotherapy.

The ability of RNAi to elicit specific transient or long-term gene knockdown has pushed the manipulation of this technique to make great strides since its discovery. As an already well-established tool to study gene function in labs today, the rate of progress has been staggering, considering the numerous technical obstacles such as delivery and integration of shRNA. The prospect of being able to inhibit gene expression to treat genetic disorders for which there is no alternative continues to drive RNAi treatments out of the theoretical and into the experimental realm.

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