RNAi technology: A Revolutionary tool for the colorectal cancer therapeutics

Wei Lv, Chao Zhang, Jia Hao

With the many changes that have taken place in people’s diet and lifestyle, colorectal cancer (CRC) has become a global concern. There were approximately 950,000 new cases diagnosed and 500,000 deaths recorded worldwide in 2000. It is the second most common type of cancer in the Western world, and it is the third most common type of digestive tumor in China. It is reported that the morbidity of CRC is 4.08/100,000 for men and 3.30/100,000 for women in China. Despite the rate of improvements in surgery, radiotherapy and chemotherapy, the overall five-year survival is around 50%. Therefore, novel treatment need to be developed in order to add to the therapeutic armamentarium.

RNA interference (RNAi) is a sequence-specific post-transcriptional gene silencing mechanism, which is triggered by double-stranded RNA (dsRNA) and causes degradation of mRNA homologous in sequence to the dsRNA. This new approach has been successfully adopted to inhibit virus replication and tumorigenicity. Recent reports have described DNA vector-based strategies for delivery of small interfering RNA (siRNA) into mammalian cells, further expanding the utility of RNAi. With the development of the RNAi technology and deeper understanding of this field, a promising new modality of treatment appeared, which can be used in combination with the existing therapies. We reviewed the proceedings on the actualities and advancement of RNAi technology for colorectal cancer therapeutics.

© 2006 The WJG Press. All rights reserved.

Key words: RNAi; Colorectal cancer; Therapeutics

Received: 2005-09-23   Accepted: 2006-01-14

INTRODUCTION

When viruses infect eukaryotic cells, or when transposons and transgenes are randomly integrated into host genomes, double-stranded RNA (dsRNA) is frequently produced from the foreign genes. Most eukaryotes, including humans, possess an innate cellular immune surveillance system that specifically responds to the presence of dsRNA and activates processes that act post-transcriptionally to silence the expression of the interloping genes[1-4]. This mechanism is now commonly referred to as RNA interference or RNAi[5]. During RNAi, long transcripts of dsRNA are rapidly processed into small interfering RNAs (siRNAs), which represent RNA duplexes of specific length and structure that finally guide sequence-specific degradation of mRNAs homologous in sequence to the siRNAs[6,7]. siRNAs may be the best tools for target validation in biomedical research today and cancer therapeutics, because of their exquisite specificity, efficiency and endurance of gene-specific silencing. Some researches about RNAi technology for colorectal cancer has been reported in recent years. However, these new approaches face serious problems before they can offer a useful adjuvant role for the clinical trials for patients with colorectal cancer.

MECHANISM of dsRNA INTERFERENCE

The key enzyme required for the processing of long dsRNAs to siRNA duplexes is the RNase III enzyme Dicer, which was characterized in extracts prepared from insect cells, C. elegans embryos, mouse cells and mast cells[8-10]. Dicer contains an N-terminal RNA helicase domain, a Piwi, Argonaute, Zwille/Pinhead (PAZ) domain[11], two RNase III domains, and a C-terminal dsRNA-binding motif. The PAZ domain is also present in Argonaute proteins, whose genes represent a poorly characterized family present in dsRNA-responsive organisms. Argonaute1 (AGO1) and Argonaute2 (AGO2), two of the five Argonaute proteins of D. melanogaster, appear to be important for forming the mRNA-degrading sequence-specific endonuclease complex, also referred to as the RNA-induced silencing complex (RISC)[12,13]. Dicer andAGO2 appear to interact in D. melanogaster Schneider 2 (S2) cells, probably through their PAZ domains; however, RISC and Dicer activity are separable, and RISC is unable to process dsRNA to siRNAs, suggesting that Dicer is not
siRNAs AS NOVEL THERAPEUTIC PLATFORM TECHNOLOGY

VEGF is one of the archetypal angiogenic growth factors and has received considerable attention. VEGF is a homodimeric 45 kDa glycoprotein, 5 different isoforms of which are reportedly expressed by endothelial cells. VEGF specifically acts on endothelial cells binding to a growing number of endothelial tyrosine kinase receptors including VEGFR-1 and VEGFR-2. Inhibition of VEGF activity or disabling the function of its receptors has been shown to inhibit both tumor growth and metastasis in a variety of animal tumor models. Given the different isoforms and their various functions, the development of this RNAi technology and its ability to target specific VEGFs should facilitate both a greater understanding of this field and also the development of improved therapeutics.

Single base pair mutations that alter the function of tumor suppressor genes and oncogenes occur frequently during oncogenesis. The guardian of the genome, p53, is inactivated by point mutation in more than 45%-60% of human colorectal cancers. Synthetic small inhibiting RNAs (siRNAs) are highly sequence-specific reagents and discriminate between single mismatched target RNA sequences, and may represent a new avenue for gene therapy. Martinez LA demonstrated that a single base difference in siRNAs differentiates between mutant and WT p53 in cells expressing both forms, resulting in the restoration of WT protein function. Therefore, siRNAs may be used to suppress expression of point-mutated genes and provide the basis for selective and personalized antitumor therapy.

The products of bel-2 genes are involved in the regulation of apoptosis and proliferation and are associated with prognosis in several malignancies, including colorectal adenocarcinoma. A statistically significant inverse association was found between Bel-2 score and tumor recurrence. It is reported that some researchers use mRNA-cDNA interference for silencing bel2 expression in human LNCaP cells. These findings indicate that a novel gene silencing system may play a useful adjuvant role in the majority of patients with colorectal cancer.

One of the major limitations of current chemotherapy regimes is the bone marrow toxicity associated with these drugs. However, it is well recognized that subpopulations of tumor cells are resistant to particular chemotherapeutic agents and continue to grow in a selective manner in the presence of such drugs. These cells contain specific genes which render them resistant to particular compounds. One such gene is the multiple drug resistance (MDR1) gene which confers resistance to vinca alkaloids (vinblastine, vincristine), anthracyclins (adriamycin, daunorubicin), etoposide and paclitaxel. For reversal of MDR1 gene-dependent multidrug resistance (MDR), two small interfering RNA (siRNA) constructs were designed to inhibit MDR1 expression by RNA interference. Some data indicate that this approach may be applicable to cancer patients as a specific means to reverse tumors with a P-glycoprotein-dependent MDR phenotype back to a drug-sensitive one.

Telomerase is an attractive molecular target toward which to direct cancer therapeutic agents because telomerase activity is present in most malignant cells but undetectable in most normal somatic cells. Koscielok BA, et al. evaluated the ability of siRNA to inhibit telomerase activity in human cancer cells. In their research, human cancer cell lines were transfected with 21 nt double-stranded RNA homologous to either the catalytic subunit of telomerase (human telomerase reverse transcriptase) or its template RNA (human telomerase RNA[hTR]). Both types of agents reduced telomerase activity in a variety of human cancer cell lines representing both carcinomas and sarcomas. Inhibition was dose-dependent. Telomerase...
inhibition by siRNA is notable because telomerase is regarded as restricted to the nucleus, whereas RNA interference is commonly regarded as restricted to the cytoplasm. Their results showed that telomerase activity in human cancer cells can be inhibited by short dsRNAs (siRNAs) targeting telomerase components. Inhibition was shown in a variety of carcinoma cell lines (HCT-15 colon carcinoma, HeLa cervical carcinoma, NCI-H23 lung carcinoma, and A431 epidermoid carcinoma).

CRC typically develops over decades and involves multiple genetic events. This has led to the development of a multistep model of colorectal tumorigenesis. In order to identify genes that are important in the development of CRC, RNAi was used to disrupt expression of two of the genes identified by microarray analysis in a colon tumor cell line, HCT116. HCT116 cells were derived from a human colon carcinoma, and showed mutations in β-catenin and K-ras, but possessed wild-type p53[32]. By examining the growth characteristics of these cells after RNAi both in vivo and in vitro, they hoped to identify targets critical for growth, apoptosis, and/or metastasis. It is clear from these results that siRNA directed against c-myc and survivin lowers the levels of these proteins without affecting the levels of a control protein, β-tubulin. Furthermore, transfection of siRNA oligonucleotides directed against both genes was as effective in reducing protein expression, as were experiments targeting each gene separately. In summary, they have demonstrated that the use of RNAi when coupled with microarray analysis provides an excellent system to define the role of specific genes that are dysregulated in cancer on both the in vitro and in vivo growth of the tumor[32].

Somatic changes in CpG dinucleotide methylation occur quite commonly in human cancer cell DNA. Relative to DNA from normal human colonic cells, DNA from human colorectal cancer cells typically displays regional CpG dinucleotide hypermethylation amid global CpG dinucleotide hypomethylation. The role of the maintenance DNA methyltransferase (DNMT1) in the acquisition of such abnormal CpG dinucleotide methylation changes in colorectal cancer cells remains obvious. Some research indicated that human colorectal carcinogenesis is accompanied by a progressive dysregulation of DNMT1 expression and suggest that abnormalities in DNMT1 expression may contribute to the abnormal CpG dinucleotide methylation changes characteristic of human colorectal carcinoma cell DNA[33]. Recently, there is a report that the controlled knockdown of DNA methyltransferase 1 (DNMT1) in human cancer resulted in growth arrest[34]. Thus the method allows for a highly controlled approach to gene knockdown.

CONCLUSION

Since biotechnologists have adopted RNAi, it has already earned a place among the major technology platforms. Despite promising data, there are several challenges that need to be faced before RNAi can be used in patients. These include mode of delivery, the precise sequence of the siRNA or shRNA used, and cell type specificity. There are possible toxicities related to silencing of partially homologous genes or induction of global gene suppression by activating the interferon response. Another potential problem is the inhibition of the function of endogenous miRNAs through competition for the RNAi machinery. Despite these hurdles, RNAi provides the opportunity to pursue an exciting new therapeutic approach to treat colorectal cancer[35].

REFERENCES

1. Hammond SM, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double-stranded RNA. Nat Rev Genet 2001; 2: 110-119
2. Hutvágner G, Zamore PD. RNAi: nature abhors a double-strand. Curr Opin Genet Dev 2002; 12: 225-232
3. Bergstrom CT, Antia R. On RNA interference as template immunity. J Biolog 2005; 30: 295-297
4. Waterhouse PM, Wang MB, Lough T. Gene silencing as an adaptive defence against viruses. Nature 2001; 411: 834-842
5. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998; 391: 806-811
6. Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev 2001; 15: 188-200
7. Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T. Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. EMBO J 2001; 20: 6877-6888
8. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. A role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 2001; 409: 363-366
9. Billy E, Brondani V, Zhang H, Müller U, Filippowicz W. Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. Proc Natl Acad Sci USA 2001; 98: 14428-14433
10. Yin D, Stuart CA. Gene silencing using small interference RNA in mast cells. Methods Mol Biol 2006; 315: 333-339
11. Cerutti L, Mian N, Bateman A. Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the Piwi domain. Trends Biochem Sci 2000; 25: 481-482
12. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 2000; 404: 293-296
13. Williams RW, Rubin GM. ARGONAUTE1 is required for efficient RNA interference in Drosophila embryos. Proc Natl Acad Sci USA 2002; 99: 6889-6894
14. Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. Science 2001; 293: 1146-1150
15. Nykänen A, Haley B, Zamore PD. ATP requirements and small interfering RNA structure in the RNA interference pathway. Cell 2001; 107: 309-321
16. Boutla A, Delidakis C, Liavaras I, Tsagris M, Tabler M. Short 5'-phosphorylated double-stranded RNAs induce RNA interference in Drosophila. Curr Biol 2001; 11: 1776-1780
17. Sijen T, Fleenor J, Simmer F, Thijssen KL, Parrish S, Timmons L, Plasterk RH, Fire A. On the role of RNA amplification in dsRNA-triggered gene silencing. Cell 2001; 107: 465-476
18. Donzé O, Picard D. RNA interference in mammalian cells using siRNAs synthesized with T7 RNA polymerase. Nucleic Acids Res 2002; 30: e46
19. Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, Conklin DS. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes Dev 2002; 16: 948-958
20. Yu JY, DeRuiter SL, Turner DL. RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proc Natl Acad Sci USA 2002; 99: 6047-6052
21. Lee NS, Dohjima T, Bauer G, Li H, Li MJ, Elsani A, Salvaterra
Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nat Biotechnol* 2002; 20: 500-505

Miyagishi M, Taira K. U6 promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells. *Nat Biotechnol* 2002; 20: 497-500

Paul CP, Good PD, Winer I, Engelke DR. Effective expression of small interfering RNA in human cells. *Nat Biotechnol* 2002; 20: 505-508

Sui G, Soohoo C, Affar el B, Gay F, Shi Y, Forrester WC, Shi Y. A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. *Proc Natl Acad Sci USA* 2002; 99: 5515-5520

Harper SQ, Davidson BL. Plasmid-based RNA interference: construction of small-hairpin RNA expression vectors. *Methods Mol Biol* 2005; 309: 219-235

Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E, Huang T, Radziejewski C, Bailey K, Fandl JP, Daly T, Wiegand SJ, Yancopoulos GD, Rudge JS. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci USA* 2002; 99: 11393-11398

Martinez LA, Naguibneva I, Lehrmann H, Vervisch A, Tchenio T, Lozano G, Harel-Bellan A. Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. *Proc Natl Acad Sci USA* 2002; 99: 14849-14854

Lin SL, Chuong CM, Ying SY. A Novel mRNA-cDNA interference phenomenon for silencing bcl-2 expression in human LNCaP cells. *Biochem Biophys Res Commun* 2001; 281: 639-644

Nieth C, Priebesch A, Stege A, Lage H. Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). *FEBS Lett* 2003; 545: 144-150

Kosciolek BA, Kalantidis K, Tabler M, Rowley PT. Inhibition of telomerase activity in human cancer cells by RNA interference. *Mol Cancer Ther* 2003; 2: 209-216

Lu X, Errington J, Curtin NJ, Lunec J, Newell DR. The impact of p53 status on cellular sensitivity to antifolate drugs. *Clin Cancer Res* 2001; 7: 2114-2123

Williams NS, Gaynor RB, Scoggin S, Verma U, Gokaslan T, Simmang C, Fleming J, Tavana D, Frenkel E, Becerra C. Identification and validation of genes involved in the pathogenesis of colorectal cancer using cDNA microarrays and RNA interference. *Clin Cancer Res* 2003; 9: 931-946

De Marzo AM, Marchi VL, Yang ES, Veeraswamy R, Lin X, Nelson WG. Abnormal regulation of DNA methyltransferase expression during colorectal carcinogenesis. *Cancer Res* 1999; 59: 3855-3860

Matsukura S, Jones PA, Takai D. Establishment of conditional vectors for hairpin siRNA knockdowns. *Nucleic Acids Res* 2003; 31: e77

Sakamoto KM. Knocking down human disease: potential uses of RNA interference in research and gene therapy. *Pediatr Res* 2004; 55: 912-913