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RNA Interference in Gastric Cancer Therapy

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Abstract

Gastric cancer is one of the most common malignant tumors, and is the second leading cause of death i from cancer worldwide. In China, gastric cancer is a high incidence cancer accounted for 42% of the world's number of gastric cancer, and is one of the highest mortality cancers in the malignant tumors [1]. Although gastroscope, laparoscopic and barium meal examination are popularized in basic-level hospitals to improve the detection rate of a certain degree of gastric cancer, and comprehensive treatment such as surgery, chemotherapy and radiotherapy has been used to therapy gastric cancer, the curative effect is not ideal for advanced gastric cancer patients due to the low resection rate, side-effects, and drug resistance. ,Therefore, the development of molecular biology and medical genetics, the discovery of tumorigenesis related genes, and new treatments of gastric cancer therapy are urgently needed. RNA interference (RNAi) is a new technology in developed rapidly in the last decade. By means of RNAi, double-stranded RNA (dsRNA) is delivered into the cells, which binds to the homologous region of the target gene and leads to the degradation of the specific mRNA, thereby inhibition the expression of the target genes. The method is efficient, fast, specific and stable to suppress excessive expression of cancer related genes, and thus becomes a hot spot in the study of tumor gene therapy. In this paper, the progress of RNAi technology in gastric cancer gene therapy was reviewed.

Keywords: RNA Interference, Gastric Cancer Therapy.

1. Introduction

The first phenomenon of RNA silencing by dsRNA (double-stranded RNA) was discovered in plant. In 1990, Napoli and Jorgensen overexpressed chalcone synthase, a key enzyme in flavonoid biosynthesis which was responsible for the deep-purple colour, in petunias. Unexpectedly, white or patchy blossoms instead of darker purple petunias were obtained [2]. In 1995, RNA silencing was first observed in animals. Introduction of sense or antisense RNA to par-1 gene in Caenorhabditis elegans leaded to the degradation of the par-1 mRNA[3]. In 1998, Fire and co-workers injected purified dsRNA and ssRNAs (single-stranded RNAs) into C. elegans, and found the inhibition effect of dsRNA was significantly higher than that of ssRNAs (sense andantisense) . And the specific gene silencing phenomenon caused by dsRNA was defined as RNA interference (RNAi) [4]. The discovery leaded a large number of groups to use RNAi to perform gene knockdown research in fruit flies, insects, plants, polyps, zebrafish, and other eukaryotes, and the expected phenomenon occurred. However, in mammalian cells introduced long dsRNA elicited a general inhibition of gene expression other than the target gene, which hampered the use of RNAi in mammalian cells. Until 2001, the chemically synthesized 21-22 nt siRNAs (small interference RNAs) were induced into mammalian cells and caused specific gene konckdown[5]. The finding heralded the use of RNAi in mammalian cells. By using chemically synthesized siRNAs, the inhibition of gene expression was time-limited since the degradation of the siRNAs. In 2002, a new vector system, named

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pSUPER, was constructed to directly synthesize siRNAs in mammalian cells and efficiently down-regulate the target gene expression stably [6]. The stable siRNAs expression system expanded the application of RNAi in a variety of mammalian cells types. In 2001, RNAi technologywas rated as one of the top ten scientific achievements by Science magazine. Fire and Mello also won the 2006 Nobel Prize in Physiology or Medicine because of their contribution in the field of RNAi research.

2. Mechanism of RNAi

At present it is generally believed that the mechanism of RNAi action consists of two phases (figure 1) [7]:

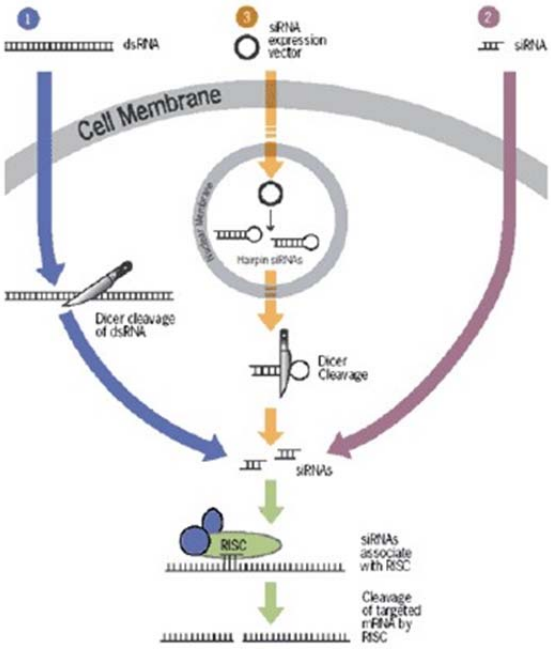

Fig. 1. The mechanism of RNA interference

(1) Initiation stage: Double-stranded RNAs generated by injection, transposon transcription or RNA virus infection are present in the cytoplasm, and cleaved by the Dicer enzyme (one of the Rnase Ⅲ endonuclease) to produce 21 ~ 23 bp (base pairs) siRNAs. These 21-23bp long dsRNAs possess a 3’ overhang of two unpaired nucleotides on each strand [8] and 5' terminal phosphate [9]. (2) Effect stage: The produced double-stranded siRNAs are wrapped up by a protein complex with nuclease activity to form the RNA - induced silencing complex (RISC) [10]. Then double-stranded siRNAs are unwound by Ago-2 and the sense component of siRNA is released. This process activates the RISC complex. Activated RISC is guided by the antisense component of siRNA to recognize and bind to the specific target mRNA, and result in mRNA degradation [11]. Alternatively, the unwound siRNA no longer acts as a guide to bring RISC to the target mRNA but serves as a primer to transform the target mRNA into a new dsRNA by an RNA-dependent RNA polymerase (RdRP). Newly synthesized dsRNAs can subsequently be recognized and cleaved by Dicer to eliminate the incorporated target mRNA and generate new siRNAs in a cycle of dsRNA synthesis and degradation [12], [13].

3. Characteristics of RNAi

RNAi technology since discovered has been the attention of scientists, and has been widely and rapidly used. This is inseparable with its unique characteristics. The main characteristics of RNAi are as follows. (1) high efficiency: In the RNAi pathway, trace amount of dsRNA can effectively inhibit the expression of target genes even to knockout through the catalytic mechanism,. (2) high specificity: The antisense strand of siRNA can specifically bind the homologous region of the target mRNA and has nothing to do with non-target genes. And only one nucleotide varied in the siRNA can lead to ineffective inhibition of the target gene. Therefore, RNAi can be used in the inhibition of a single nucleotide mutation gene expression [14]. (3) Diffusion and heritability: siRNA can be diffused from the injected/infected cells to other cells. Intravenous injection of Fas siRNA specifically reduced Fas gene expression in mouse hepatocytes [15]. In C. elegans, gonadal injection of dsRNA can result in offspring to inhibit the target gene expression. (4) Rapid reaction: dsRNA can rapidly induce target mRNA degradation. In drosophila embryos, the mRNA expression level of the target gene was dramatically decreased in 30min after the dsRNA injection [16]. (5) Stability: siRNA can be stable in 3-4 d within the cells, and the half-life is higher than that of antisense RNA. (6) Length-dependency, ATP-dependent, simple operation and other characteristics.

4. Application of RNAi in gastric cancer therapy

4.1 Role in gastric cancer related gene research

Gastric tumorigenesis is a complicated process, which is integrated with a variety of genetic and environmental factors. Through activation of oncogenes and inactivation of tumor suppressor genes, it results in a multi-stage, multi-step process, eventually leads to the malignant cell proliferation and apoptosis. RNAi can specifically act on the target genes by preventing the activation of oncogenes and the inactivation of tumor suppressor genes to achieve the goal of treatment of gastric cancer. Compared with conventional gene therapy, RNAi can simultaneously interfere with multiple target genes to furthest prevent tumor development. Since RNAi technology was discovered, it has been widely studied in tumor gene therapy owing to its unique advantages.

NOL8 was focused as a target gene in diffuse-type cancers since it was specifically overexpressed in diffuse-type cancer cells. Transfection of siRNA specific to NOL8 into three diffuse-type gastric cancer cells, St-4, MKN45 and TMK-1, could significantly reduce NOL8 expression and induce apoptosis in these cells [17]. Bcl-2 plays a role in regulation of cell apoptosis, and it is overexpressed in the gastric carcinoma.Transfection of the human gastric cancer cells SGC-7901 with Bcl-2 siRNA could significantly inhibit Bcl-2 expression and suppress the growth of gastric cancer cells. Also, Bcl-2 siRNA could decrease telomerase activity [18]. Survivin is a widely expressed apoptosis inhibitor in many cancer cells, and plays a role in the regulation of cell division. Downregulation of surviving expression in the SGC-7901 cells by RNAi could inhibit the tumor cell growth and induce apoptosis of SGC-7901 cells [19]. Polo-like kinase 1 (PLK-1) is an important protein in MAPK signal pathway, and overexpressed in lots of tumor cells. Inhibition of plk1 expression in the gastric cancer cell line-MKN45 cells by specific siRNA could increase apoptosis in the cells and reduce tumor cells proliferation [20]. P42.3 is a specifically expressed gene in gastric cancer tissuebut not in normal gastric mucosa tissues, and is associated with M-phase regulation. Inhibition of p42.3 expression in gastric cancer cell line-BGC823 by RNAi technique found that significant suppression of cell proliferation and tumorigenicity happened. [21]. Heat shock protein (HSP) 70 might play important role in malignant transformation and

maintenance of malignant phenotypes in gastric cancer. Suppression of Hsp70 expression in gastric cancer cell line BGC823 using RNAi technology could effectively inhibit growth of cancer cells, induce cell cycle arrest and increase cell apoptosis in vitro and in vivo[22] . Inhibitor of DNA binding/differentiation 1 (ID1) is the factor of multiple signaling pathways regulating the activation energy to increase VEGF expression, and promote the proliferation of tumor cells. Lei, etc. [23] through the RNAi, according to the results of ID1 mRNA and protein expression decreased, The tumor weight of the nude mice injected with human gastric cancer cells SGC7901 which transfected with ID1 specific siRNA was significantly lower than that of control group. This proved that ID1 could promote the proliferation of tumor.

Utilization of RNAi technique, more and more new oncogenes and tumor suppressor genes were discovered, and a deeper understanding of the interactions between various factors in the occurrence and proliferation of gastric cancer was further illuminated. Thus, RNAi technique brings bright prospects in gastric cancer gene therapy.

4.2 Role in inhibiting tumor blood vessels and lymphatic hyperplasia in treatment of gastric cancer

Tumor cells division, tumor growth and metastasis depend on angiogenesis, thus inhibition of angiogenesis can be a method of treatment of malignant tumors. Vascular endothelial growth factor (VEGF) is a key factor to stimulate vasculogenesis and angiogenesis, and it can be specifically act on endothelial cell mitogen. VEGF plays an important role in tumor angiogenesis, therefore knockdown VEGF by RNAi can effectively inhibit tumor angiogenesis and growth. Xu Wenhua etc. [24] using RNAi technology, designs transfection After human gastric cancer cell line SGC-7901 transfected with two groups of siRNA targeting VEGF, both of the mRNA and protein expression levels of VEGF were notably decreased. Suppression of VEGF expression effectively inhibited the proliferation of human gastric cancer cells [24]. Rac1 is a prominent member of the Rho family and is critical in regulating hypoxia-induced gene activation of several angiogenesis factors and tumor suppressors. When knockdown Rac1 expression in gastric cancer cell line AGS by RNAi technique, the condition medium derived from the Rac1 knockdown AGS cells could significantly inhibit the human endothelial cells proliferation. Moreover, two angiogenesis promoting factors, VEGF and hypoxia induced factor (HIF) -l alpha, were also downregulated in the Rac1 knockdown AGS cells whereas the expressions of two tumor suppressors and angiogenesis inhibitors, p53 and VHL, were increased. This result indicated that Rac1 could regulate a correlation factor of angiogenesis, and promote the generation of blood vessels in tumor cells [25]. When gastric cancer cells SGC7901 were transfected with a Raf-1 specific siRNA vector, the expression of Raf - 1, VEGF and HIF -l alpha was dramatically decreased. Apoptosis in Raf-1 knockdown cells was increased and cellular proliferation in these cells was decreased [26]. Knockdown cyclooxygenase-2 (COX-2) expression in SGC7901 cells could inhibit Id1 and VEGF expression, and COX-2 could stimulate VEGF and enhance the proliferation of endothelial cells by upregulating Id1. So COX-2 and Id1 can be exploited as new gene therapeutic targets of gastric cancer [27], Lymphoid hyperplasia facilitates transfer of tumor cells. VEGF - C plays an important role in stimulating growth of lymphatic endothelial cells and lymphatic vessel formation. In gastric cancer and other malignant invasive tumors, high expression of VEGF - C can cause lymph node metastasis of tumor. Transfectionof SGC - 7901 cells with constructed siRNA expression vector specific targeting VEGF-C could significantly inhibit VEGF - C expression [28]. And SGC-7901 cells transfected with VEGF-C siRNA

expression vector dramatically suppressed tumor lymph angiogenesis, tumor growth and regional lymph-node metastasis in subcutaneous xenografts [29].

4.3 Role in gastric cancer drug resistance study

Chemotherapy is one of the main means of treatment of gastric cancer, and drug resistance of tumor cells often causes the failure of chemotherapy. Drug resistance of tumor cells is related to some resistance genes, and specifical knockdown certain resistance related gene by RNAi might improve the effect of chemotherapy. Multi-drug resistance (MRD), a major cause of failure to provide effective chemotherapy to many patients, is usually associated with the overexpression of P-glycoprotein (P-gp) [30]. P-gp is coded by the multidrug resistance 1 (MDR1) gene, and is responsible for decreased drug accumulation in multidrug-resistant cells. When PGP abnormally expressed in the cells, constitutive PGP expression will reduce drug concentration in the cells, and meanwhile inhibit caspases apoptosis pathway, which causes the cells to be drug resistant [31]. Transfection of extremely high drug-resistant human gastric carcinoma cell line EPG85-257RDB with the anti-MDR1/P-gp shRNA expression vectors showed that the cells resistant to tumor drug soft erythromycin were reduced by 58%. The result indicated that stable shRNA-mediated RNAi could tremendously reverse MDR1/P-gp-mediated MDR and therefore be a promising strategy for overcoming MDR by gene therapeutic applications [32]. ZNRD1 (zinc ribbon domain containing 1) can promote multidrug resistance in gastric cancer cells by activating MDR1 expression. When SGC7901 cells were transfected with the ZNRD1 siRNA expression vector, suppression of ZNRD1 expression significantly enhanced the sensitivity of SGC7901 cells to vincristine, adriamycin and etoposide, which indicated inhibition of ZNRD1 expression could effectively reverse the resistant phenotype of gastric cancer cells [33]. Tumor susceptibility gene 101 (TSG101) is overexpressed in vincristine-resistant human gastric adenocarcinoma cells. Suppression TSG101 expression in SGC7901 cells using RNAi significantly enhanced the sensitivity of SGC7901 cells to vincristine and Adriamycin [34]. C-terminal binding protein 1 (CtBP1) is a transcriptional co-regulator that participates in transcriptional activation of the MDR1 gene. Knockdown CtBP1 expression in human multidrug resistant cancer cell lines decreased MDR1 expression, and enhanced the sensitivity of MDR cells to some chemotherapeutic drugs. Thus, CtBP1 might be a new target for inhibiting drug resistance [35]. Cytokine-induced apoptosis inhibitor 1 (CIAPIN1), an apoptosis inhibitor, is highly expressed in multidrug resistant gastric cancer cells. Inhibition of CIAPIN1 expression in SGC7901 cells decreased MDR1 and MDR-related protein-1 (MRP-1) expression, and sensitized SGC7901 cells to anticancer drugs [36]. Telomeric repeat-binding factor 2 (TRF2) plays a key role in the protective activity of telomeres. Knockdown TRF2 in SGC7901 cells by RNAi partially reversed drug resistance phenotype, which indicated TRF2 was involved in drug resistance of gastric cancer [37].

5. Problems and prospects

Since RNAi was discovered, much attention has been paid to improve the technology and a set of mature molecular biology methods have been established. However, there are still some problems to be solved before RNAi are widely used in clinic. When siRNA is introduced into the human body, there are lots of unpredictable questions, such as, whether the effect in human body is consistent with the in vitro experiments and animal model, and whether there is a difference between different individuals. The technology application in human body must be carefully considered the stability of siRNA, the efficient

effect and low toxicity for human body, and so on. Advances in understanding of the mechanisms of RNAi will improve the application in cancer therapy [38].

Incidence of gastric cancer is associated with a variety of carcinogenic factors and tumor-suppressor gene. Utilization of RNAi technology to knockdown these key factors expression can inhibit tumor cell proliferation, invasion and drug resistance, thus to achieve the goal of treatment of tumor. Recently, RNAi has been used to study gastric cancer development, aspects and vaccine for stomach cancer tumor. With the constant improvement of RNAi technology, it will become a new direction of the gastric cancer gene therapy.

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