RNA activation technique and its applications in cancer research

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Abstract: RNA activation (RNAa) is a mechanism of gene activation mediated by small activating RNAs. The activation of gene expression by small activating RNA has excellent targeting specificity and flexibility, with a persistent and strong effect. Studies have shown that the RNAa technique has broad prospects for application in the research on tumor pathogenesis and the treatment of tumors. This paper reviews the literature on RNAa with regard to the course of discovery, the mechanisms and characteristics of action, and the current status and prospects of application.

Keywords: RNA activation, small activating RNA, cancer

Introduction

RNA activation (RNAa) is a mechanism of gene activation mediated by various small RNAs, known as small activating RNAs (saRNAs), including double-stranded RNA (dsRNA), microRNA (miRNA), and piwi-interacting RNA. By targeted binding to a specific gene promoter region or antisense transcript, saRNA can regulate target gene transcription through epigenetic mechanism. In addition, saRNA can directly promote target mRNA translation or antagonize the binding of miRNA to the 3'-untranslated region (3'-UTR) of a target mRNA, thereby enhancing post-transcriptional gene expression. saRNA has unique patterns and characteristics of gene expression, and its intervention of gene expression shows excellent targeting specificity and flexibility in the selection of target gene and target site, with a persistence and strong effect [1-6]. Studies have shown the RNAa technique has broad prospects for application in the research on tumor pathogenesis and the treatment of tumors [7-10]. This paper reviews the literature on RNAa with regard to the course of discovery, the mechanisms and characteristics of action, and the current status and prospects of application.

Course of discovery of RNAa

In 1969, Britten et al. [11] found that some non-coding RNAs were capable of specifically recognizing DNA and binding multiple proteins to activate the expression of a large group of genes. These RNAs were referred to as saRNAs and their activation of gene expression was believed to be dependent on the complementarity between base pairs. This was the earliest study to report the activation of gene expression by RNA. However, due to cellular difference in the activation, there were great difficulties in designing RNA molecules for universal activation, so no attention was received. In 2002, Kuwabara et al. [12] found that the neuron restrictive silencer element/repressor element-1 dsRNA isolated from human hippocampal neural stem cells was able to bind to the neuronal restricted silencing factor/RE-1 silencing transcription factor (NRSF/REST) and relieve the suppressed state of differentiation-inducing genes in neural stem cells, resulting in the differentiation of neural stem cells into differentiated cells such as neuronal and glial cells. In 2005, Jopling et al. [13] found that miR-122, which was highly expressed specifically in human hepatocytes, could bind to and interact with the 5'-non-coding region of the hepatitis C
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virus (HCV) genome, significantly enhancing the proliferation ability of HCV; in contrast, the autonomous replication of HCV markedly decreased after the blocking of miR-122. In 2006, Li et al. [14] designed 21-bp dsRNAs targeted to epithelial cadherin (E-cad), tumor suppressor gene p21, and vascular endothelial growth factor (VEGF), which were shown to up-regulate the expression of these target genes in a variety of human tumor cells and normal cells. In 2007, Janowski et al. [15] proved again in human breast cancer cells that dsRNA could up-regulate the expression of progesterone receptor (PR). Subsequently, several other research groups also reported similar results, showing the existence of the RNAa phenomenon in mammalian cells of primates including humans, as well as rats and mice [16].

Mechanisms of action of RNAa

So far, the mechanisms of action of RNAa have not been completely understood. It has been reported that saRNA can target promoter-specific sequence to alter the position and spatial conformation of certain binding proteins around the promoter, which further induces DNA or histone modification, thereby activating target gene expression. Moreover, saRNA can act on an antisense transcript and weaken its silencing effect on a target gene, playing a role in gene activation. Meanwhile, dsRNA can also enhance post-transcriptional gene expression by directly promoting target mRNA translation or antagonizing the binding of miRNA to the 3'-UTR of a target mRNA [1-10].

saRNA targets promoter-specific sequence and activates gene expression via epigenetic mechanisms

Li et al. [14] designed 21-bp dsRNAs targeted human E-cad, p21, and VEGF gene promoter (excluding high GC regions such as CpG islands and Alu repeats). The expression of these target genes was found to be specifically and persistently (2-13 d) activated by dsRNAs in human breast cancer MCF-7 cells, human cervical cancer HeLa cells, human prostate cancer PC-3 and LnCap cells, human bladder cancer J82 and T24 cells, and human embryonic kidney HEK293 cells. Furthermore, these researchers transfected PC-3 cells with a small interfering RNA specifically targeted to the promoter region of E-cad and having proven to silence its expression. The transfection resulted in a significant decrease of E-cad levels in the cells, suggesting that dsRNA-mediated RNAa was target-specific and dependent. Meanwhile, the activation effect on E-cad and p21 was completely lost after substituting five base pairs in the 5’-end of the antisense nucleic acid strand in the dsRNA, but no marked changes occurred in the activation effect after substituting the same number of bases in the 3’-end and the middle part. This suggested that the 5’-end of the dsRNA sequence was a key part for RNAa. These researchers designed small interfering RNAs targeted to the argonaute (Ago) family (siAgo1-siAgo4) and transfected target cells. After the silencing of Ago1, Ago3, and Ago4, dsp21-322 still up-regulated p21 expression, whereas after the silencing of Ago2, the activation effect of dsp21-322 completely disappeared. Therefore, Ago2 was considered to play a decisive role in the activation by dsRNA. In addition, these researchers found that during the process of RNAa, a reduction in the level of histone H3 lysine-9 (H3K9) methylation was closely related to the occurrence of RNAa. Li et al. [14] also found that dsRNA was unable to induce DNA methylation. This result was consistent with the conclusion of Li et al. [17] with regard to RNAa in human lung cancer A549 and H292 cells, where dsRNA promoted the up-regulation of tumor suppressor gene PTEN expression, without changing the methylation level in the promoter region of the PTEN gene.

Janowski et al. [15] designed dsRNAs targeted to the promoter region of human PR gene and selected PR11 dsRNA for transfection of human breast cancer T47D and MCF-7 cells based on the experimental results. The results showed that dsRNA significantly up-regulated PR protein and mRNA expression levels. In addition, a one base-pair change in PR11 led to the loss of its activation effect, which further proved the high sequence specificity of dsRNA used for gene activation. Moreover, the duration of RNAa lasted up to 15 d or more, but the activation effect gradually declined with increasing time and number of cell passages. Mechanism studies failed to prove the important role of Ago in RNAa; nonetheless, these researchers found that the RNAa process was associated with a decrease in histone H3K9 and H3K14 acetylation and an increase in histone H3K4 dimerization and trimethylation.
Through bioinformatics analysis, Huang et al. [9] found that multiple miRNA-binding sites were present in the promoter region of cyclin B1. On this basis, dual-luciferase reporter assays showed that miR-744, miR-1186, and miR-466d-3p induced cyclin B1 expression in mouse NIH/3T3 cells, while the blocking of endogenous miR-744 significantly reduced cyclin B1 expression. These results proved that miRNA could up-regulate gene expression. Functional assays showed that overexpression of miR-744 and miR-1186 significantly up-regulated cyclin B1 expression level and promoted cell proliferation in mouse prostate cancer TRAMP C1 cells; however, after long-term expression, the resulting chromosomal instability and other factors led to gene silencing and inhibition of tumor growth. Furthermore, mechanism studies of chromatin immunoprecipitation showed that Ago1, RNA polymerase II, and trimethylated histone 3 at lysine 4 (H3K4me3) played key roles in miRNA-mediated up-regulation of cyclin B1 expression.

**saRNA acts on antisense transcript and weaken its silencing effect on target gene**

Morris et al. [18] found that an antisense transcript capable of binding to the antisense strand in the vicinity of the p21 gene promoter region blocked positive mRNA expression of the sense strand through recruiting Ago1 and H3K27me3 to the promoter region of the sense strand, making the gene enter a silenced state with a low expression level. After the unwinding of double-stranded saRNA, the antisense strand formed a complex with the Ago2 protein and then bound to the antisense transcript of the p21 gene. Thus, the effect of antisense transcripts in recruiting Ago1 and H3K27me3 to inhibit mRNA transcription of the sense strand was reduced, which further enhanced the sense expression of p21 mRNA and thereby achieved gene activation. Schwartz et al. [19] further proved that the antisense transcript of a target gene was the real target molecule of saRNA for gene activation, based on a study of transcriptional regulation in the promoter region of the PR gene. These results indicate that saRNA can act on an antisense transcript and weaken its silencing effect on a target gene, leading to up-regulation of gene expression.

**saRNA promotes mRNA translation and enhances post-transcriptional gene expression**

Vasudevan et al. [20] found that human pre-miR369-3 induced the binding of Ago2, fragile X mental retardation-related protein 1, and factors associated with micro-ribonucleoproteins to the AU-rich element at the 3'-end of tumor necrosis factor-α (TNFα) mRNA, which activated TNFα mRNA translation in the stationary phase of cell cycle and thus up-regulated TNFα expression. Moreover, Let-7 miRxcx4 was shown to play a similar role as pre-miR369-3 in activating target mRNA translation in stationary-phase cells, although the former had proven to inhibit target mRNA translation in proliferating cells. These results suggest that saRNA can achieve post-transcriptional activation of the target gene by directly facilitating mRNA translation.

**saRNA antagonizes the recognition and binding of miRNA to mRNA 3’-UTR and enhances post-transcriptional gene expression**

Through luciferase reporter gene assays and point mutation assays, Yu et al. [21] demonstrated that miR-205 decreased SHIP2 mRNA and protein expression levels in Hela cells and primary human epidermal keratinocytes by targeted binding to the 3’-UTR of SH2-containing inositol polyphosphate 5-phosphatase (SHIP2) mRNA. However, the down-regulatory effect of miR-205 on SHIP2 expression was reversed by the addition of miR-184 into Hela cells, suggesting that miR-184 could antagonize miR-205 and play a role in the maintenance of SHIP2 expression level. Further analysis showed that miR-205 was highly expressed in highly malignant squamous cell carcinoma SCC68 and CAL27 cells, while SHIP2 showed low expression. The proportion of apoptotic and necrotic cells markedly increased in these cells after transfection with a miR-205 antagonist, antagomir or miR-184, which was closely related to a significant increase of SHIP2 expression and an inactivation of pAkt and p-BAD. Thus, these researchers have elucidated a new mechanism of RNAa that miRNA can improve post-transcriptional gene expression level by antagonizing the binding of other miRNAs to the 3’-UTR of target mRNA.
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Targeting E-cadherin

E-cadherin is a calcium-dependent cell adhesion molecule, whose expression down-regulation or loss can enable the cells to obtain an invasive ability and undergo dissociation and shedding, facilitating the occurrence of epithelial-mesenchymal transition [22, 23].

Mao et al. [24] transfected the human bladder cancer cell line 5637 with an E-cad dsRNA designed by Li’s team. They found that dsRNA induced an increase in E-cad expression while inhibiting cell migration and invasion. As shown by mechanism studies, this result was related to β-catenin relocation from the nucleus to membrane and a reduction of β-catenin-mediated transactivation. The role of dsRNA in E-cad activation and its effect on the biological behavior of tumor cells were further demonstrated by Wei et al. [23]. These researchers found that the proliferation ability and clonality of human breast cancer MCF-7 and MDA-MB-453 cells significantly decreased after transfection with dsEcad-215, while the proportions of apoptotic cells and G2/M-phase cells markedly increased. This process was associated with an inhibition of the anti-apoptotic gene survivin expression. Meanwhile, the transfection of dsEcad-215 considerably reduced cell migration ability. In a nude mouse model established with MDA-MB-453 cells, dsEcad-215 was found to inhibit tumor growth in vivo. These results suggest that dsRNA-mediated E-cad gene activation can inhibit the proliferation, invasion, and migration of tumor cells, and thus has a potential antitumor effect.

Targeting p21

Chen et al. [25] transfected human bladder cancer T24 and J82 cells with a dsRNA designed by Li’s team, which was targeted at site 322 (transcription initiation site as 0) of the cell cycle arrest protein p21 gene promoter. The results showed that p21 expression markedly increased at both mRNA and protein levels, leading to a considerable reduction in cell proliferation ability and clonality, while the proportion of apoptotic cells and G1-phase cells significantly increased. In addition to inhibiting cell proliferation, RNAa-mediated p21 gene activation also increased the chemosensitivity of tumor cells. Wei et al. [26] also transfected human lung cancer A549 cells with the above-mentioned dsRNA and found that dsp21 up-regulated p21 mRNA and protein expression levels while significantly inhibiting cell proliferation. More importantly, dsp21 increased the chemosensitivity to cisplatin in A549 cells. In vivo experiments showed that dsp21 inhibited tumor growth in a xenograft nude mouse model of lung cancer. Moreover, Whitson et al. [27] and Kosaka et al. [28] found that dsp21 respectively induced tumor cell apoptosis and cell cycle arrest in renal cancer cells and liver cancer. Recently, Li’s team injected dsp21 as lipidoid-encapsulated nanoparticles into xenograft mice with prostate cancer [29] and bladder cancer [30]. These studies showed that dsp21 significantly inhibited tumor growth, further proving the potential value of dsRNA for application in antitumor therapy. Zhang et al. [31] designed dsp21 (saRNA) targeted to p21-WAF1/CIP1 using the RNAa technique. The dsp21 activated p21WAF1/CIP1 gene expression, inhibited cell proliferation, and induced apoptosis in lung cancer H441 cells, which provided a new thinking and method for gene therapy of lung cancer.

Targeting Krüpple-like transcription factor KLF4

Wang et al. [32] synthesized several dsRNAs targeting Krupple-like factor 4 (KLF4) according to the designing principle of saRNA and then transfected human prostate cancer PC-3 cells. Both dsKLF4-496 and dsKLF4-525 were shown to significantly up-regulate KLF4 expression level. Meanwhile, these two dsRNAs led to a significantly decrease in cell proliferation ability and clonality, and a significant increase in the proportion of G1/G and G2/M phase cells. This suggested that dsRNA-mediated up-regulation of KLF4 could inhibit the growth of tumor cells. Mechanistic studies showed that these effects were related to changes in the expression of a series of cell cycle-related proteins after KLF4 up-regulation, such as p21, p27, cyclin B1, centromere protein E, mitotic arrest deficient 2-like 1, and budding uninhibited by benzimidazoles 1 homolog B, etc. Moreover, Wang et al. [32] also repeated the experiments using a vector-based overexpression technique and the results were consistent with those from RNAa-mediated
overexpression experiments. This has confirmed the reliability and stability of the RNAa effect, and also indicated that RNAs can be used to activate specific genes for experimental studies of gene overexpression in tumors.

**Targeting Wilms’ tumor gene 1 (WT1)**

Wilms’ tumor gene 1 (WT1) was first cloned and identified as a tumor suppressor gene in nephroblastoma (also known as Wilms’ tumor) and localized to 11p13. The coding product of WT1 gene is a zinc finger transcription factor that has dual functions of transcriptional activation and suppression. During the development process, WT1 participates in the formation of organs such as heart, kidney, and spleen by regulating multiple target genes and signaling pathways. Studies have shown that abnormal WT1 expression in adult tissues is closely related to the occurrence of various malignant tumors, but it may act as an oncogene or tumor suppressor gene depending on cell characteristics [33-39]. A recent study by Qin et al. [40] showed that dsWT1-319 up-regulated WT1 mRNA and protein expression levels in human liver cancer HepG2 cells. In addition, dsWT1 significantly inhibited cell proliferation ability and the effect was dose- and time-dependent. Moreover, dsWT1 also significantly inhibited the number and volume of cell clones formed, while it increased the proportion of apoptotic cells. Mechanism studies showed that the above effects were related to decreased expression of anti-apoptotic proteins Bcl-2 and pro-caspase-3 and increased expression of proapoptotic proteins Bak and caspase-3, and the DNA repair enzyme poly ADP-ribose polymerase. These findings imply that RNAa-mediated WT1 activation may be a novel therapeutic strategy for cancer [40, 41].

**Targeting tumor suppressor gene p53**

The transcription factor p53, an important tumor suppressor protein encoded by human TP53 gene, is capable of maintaining genomic integrity and preventing cellular DNA damage and tumor formation by selectively modulating DNA repair, cell cycle arrest, senescence, and apoptosis [42]. p53 is the most common mutant gene in human malignancies, and is therefore an important target of tumor gene-specific therapy [41]. It has been reported that the decrease of p53 expression is related to the development process of prostate cancer cells [43]. Ge et al. [44] used dsP53-285 to suppress prostate cancer cells by regulating the expression of wild-type p53. It was experimentally proved that dsP53-285 significantly activated wild-type p53 expression by targeting the p53 promoter region in human prostate cancer cell lines LNCaP and DU145, whereas overexpression of p53 down-regulated the expression of cyclin D1 and cyclin-dependent kinase (CDK) 4/6, induced G0/G1 phase cell cycle arrest in prostate cancer cells, and inhibited cell proliferation and clone formation. Subsequently, Ge et al. [44] and Wang et al. [45] found that dsP53-285 activated p53 expression in human bladder cancer cells T24 and EJ by acting on the p53 promoter region. In vitro experiments showed that dsP53-285 inhibited cell proliferation and clone formation, induced G0/G1 phase cell cycle arrest, and inhibited cancer cell invasion and metastasis. The expression of cyclin D1 and CDK 4/6 was down-regulated in human bladder cancer cells transfected with dsP53-285, while the expression of epithelial-mesenchymal transition-related genes (E-cad, β-catenin, ZEB1, and vimentin) was elevated. In vivo experiments in nude mice demonstrated that dsP53-285 significantly inhibited the xenografts and metastases of bladder cancer.

**Targeting VEZT**

The VEZT gene is a new tumor suppressor gene located on chromosome 12q22, encoding a key plasma membrane protein that is involved in cell adhesion and junction. Xie et al. [46] used saRNA-3 to act on the promoter region of the VEZT gene in gastric cancer cells SGC-7901 and M-28. It was found that saRNA-3 significantly up-regulated the expression of VEZT gene and thereby inhibited the growth, invasion, and metastasis of gastric cancer cells.

**Targeting C/EBPα**

C/EBPα, encoded by the transcription factor CCAAT/enhancer-binding protein-α gene, belongs to the C/EBPα transcription factor family. C/EBPα is a basic region leucine zipper transcription factor. As a tumor suppressor protein, C/EBPα can cause mitotic arrest by activating p21 and inhibiting E2F and CDK [47]. Studies showed that C/EBPα expression was down-regulated in liver cancer compared with normal
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liver, and that C/EBPα played an important role in inhibiting the proliferation of HepG2 cells [48, 49]. Subsequently, Reebye et al. [48] used saRNA to act on C/EBPα and found an increase of more than 2-fold in C/EBPα and albumin transcription levels, whereas the elevated C/EBPα expression successfully reduced tumor load and improved clinically relevant liver cirrhosis or liver dysfunction in a hepatocellular carcinoma model. In addition, C/EBPα saRNA inhibited tumor growth of liver cancer in a mouse model of hepatocellular carcinoma. Later, Voutila et al. [50] also experimentally proved that CEBPA-51 (saRNA) up-regulated CEBPA mRNA expression in human hepatocellular carcinoma cells by 2.5-fold. The enhancement of C/EBPα activity not only improved liver function, but also inhibited the growth of hepatocellular carcinoma. C/EBPα was currently undergoing phase I clinical trials for patients with liver cancer. Sorah et al. [51] linked C/EBPα-saRNA with 2'-fluoropyrimidine RNA to form cell-specific transport vectors P19-C/EBPα-saRNA and P1-C/EBPα-saRNA, which enhanced C/EBPα expression and significantly inhibited cell proliferation in pancreatic ductal adenocarcinoma. In addition, it was also proved by xenografting in mouse pancreatic ductal adenocarcinoma that P19-C/EBPα-saRNA could inhibit tumor growth.

Target HIC1

The hypermethylated in cancer 1 (HIC1) gene is a tumor suppressor gene localized to 17p13.3. HIC1 is a transcriptional repressor with 714 amino acids, containing five Krüppel-like C2H2-type zinc-finger motifs and an N-terminal BTB/POZ domain with ~130 amino acids [52]. HIC1 can promote tumorigenesis in a variety of cell types, such as the breast [53]. Pan et al. [54] also demonstrated that HIC1 gene expression level in gastric cancer tissues and cells was significantly lower than that in normal tissues. Subsequently, these researchers used dsHIC1-2998 to act on the promoter region of the HIC1 gene, which increased HIC1 mRNA levels by 4.5- and 6.4-fold in gastric cancer cells MKN-28 and BGC-823, respectively, thereby inhibiting cell proliferation, colony formation, and cell metastasis, and inducing cell cycle arrest in gastric cancer cells. Moreover, the total apoptotic rate significantly increased in the in gastric cancer cells SGC-7901. Thereafter, Pan and Zhao et al. [55, 56] also found that HIC1 gene expression was significantly down-regulated in breast cancer cells. HIC1 mRNA level respectively increased by 2.2- and 5.7-fold in MCF-7 and MDA-MB-231 cells after transfection with dsHIC1-2998. The up-regulation of HIC1 not only inhibited cancer cell growth, clone formation, invasion, and metastasis, but also induced cell apoptosis and cell cycle arrest. HIC1 is therefore a potential target of gene therapy for gastric cancer, while dsRNAs also provide a new option for the up-regulation of tumor suppressor genes in various malignancies.

Targeting NKX3-1

The NK3 homeobox 1 (NKX3-1) gene localized to human chromosomal region 8p21 [57] is a prostate-specific tumor suppressor gene [58]. Studies have shown that NKX3-1 expression is often lost or down-regulated [57, 59], while mutations have rarely been found [60]. Restoration of NKX3-1 function by vector-mediated ectopic expression can inhibit in vitro prostate cancer cell proliferation and in vivo tumor growth [61]. Ren et al. [62] selected dsNKX3-1-381 targeting the promoter region of human NKX3-1 for in vitro transfection of human prostate cancer cells (LNCaP, CWR22R, PC-3, CWR22RV1, DuPro, LAPC4, and DU145). It was found that NKX3-1 mRNA levels increased by 1.5-6.4 fold in different cancer cells 96 h after transfection. Moreover, after transfection with dsNKX3-1-381, NKX3-1 possibly mediated the up-regulation of p21 and p27 gene expression and the inhibition of VEGFC gene expression, leading to proliferative inhibition, slow growth, increased cell death, cell cycle arrest, and apoptosis in prostate cancer cells. Mouse xenograft experiments showed that NKX3-1 activation significantly inhibited tumor growth in prostate cancer and prolonged the survival time in animals. This study also verified the potential of saRNA-targeted activation of tumor suppressor genes in the treatment of prostate cancer.

Targeting PAWR

The human PRKC apoptosis WT1 regulator (PAWR) gene, also known as PAR4 or Par-4 gene, is located on chromosome 12q21 and encodes a leucine zipper domain protein [63, 64]. A study has shown that overexpression of ectopic PAWR induces apoptosis in most can-
cer cells and inhibits tumor growth of prostate cancer [65]. PAWR is therefore an important tumor suppressor gene in apoptosis and a selective target of cancer therapeutic agents. Yang et al. [10, 66] found that dsPAWR-435 significantly increased PAWR expression in human bladder cancer cell line T24 and there was a more than 3-fold increase in PAWR mRNA and protein expression. Subsequently, these researchers reached the same conclusion when targeting dsPAWR-435 to PAWR in prostate cancer cells. In addition, further experiments revealed that dsPAWR-435 inhibited the growth of prostate cancer cells and induced apoptosis in cancer cells. Activation of PAWR by saRNA may provide a new therapeutic method for prostate cancer and other cancers.

Targeting DPYSL3

The human dihydropyrimidinase-related protein 3 (DPYSL3) gene, also known as collapsin response mediator protein 4 (CRMP4) gene, is a member of a gene family that encodes five DPYSLs related to semaphorin- and collapsin-mediated cellular responses [67]. Gao et al. [68] found that DPYSL3 (CRMP4) protein acts as a tumor metastasis suppressor in prostate cancer. In a study exploring new strategies for inhibition of tumor metastasis, Li et al. [69] found that the treatment of saRNA in the promoter region of the DPYSL3 gene in prostate cancer cells significantly increased DPYSL3 expression. Moreover, in vitro experiments showed a significant reduction of cell invasion and metastasis by saRNA in prostate cancer cells PC-3 and C4-2, whereas distant metastasis of prostate cancer was also significantly inhibited by saRNA in an orthotopic xenograft mouse model.

Targeting PTEN

The phosphatase and tensin homologue deleted on chromosome 10 (PTEN) gene is located on chromosome 10q23 and encodes a multifunctional protein with 403 amino acids. PTEN acts as a suppressor gene that plays a role in a variety of malignant tumors [70]. Li et al. [17] found that PTEN expression in lung cancer cells was significantly lower than that in normal tissues, whereas PTEN gene expression was elevated in lung cancer cell lines A549 and H292 after transfection with dsPTEN. These researchers have suggested that saRNA can be used to activate PTEN gene expression in cancer cells for inhibiting tumor growth.

Conclusion and prospects

At present, the mechanisms of action of RNAa have not been fully understood, the design method of saRNA has not been completely mastered, and further exploration is being needed. Nonetheless, given the designability of saRNA and its specific, long-lasting, and universal effects, saRNA has outstanding advantages in basic research and treatment of tumors. It is believed that in the future, with further research on RNAa, we will learn more phenomena about RNAa and reveal the mechanisms of action of RNAa. On this basis, we can overcome the difficulties in the application of RNAa, develop the method for in vivo treatment of clinical cancer by saRNA, and expand new fields of cancer treatment, enabling its wide use in the treatment of tumors.

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Disclosure of conflict of interest

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