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Mini-review

RNA activation: Promise as a new weapon against cancer

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ABSTRACT

RNA activation (RNAa) is a novel mechanism in which short RNA duplexes, referred to as small activating RNAs (saRNAs), enable sequence-specific gene activation capable of lasting up to 2 weeks. RNAa was named in contrast to RNA interference (RNAi). Although many mysteries remain, increasing evidence demonstrates that RNAa not only provides a novel mechanism for the study of gene function and regulation, but also holds exciting potential for clinical translation to therapeutic modality against cancers. In this review, we will focus on the potential applications of RNAa in cancer studies and therapeutics.

1. Introduction

RNA activation (RNAa) is a recently discovered gene regulation mechanism mediated by small double-strand RNA (dsRNA) that targets gene regulatory sequences (e.g. promoter as well as certain other non-coding regions), and involves transcriptional and epigenetic alterations. RNA interference (RNAi) and RNAa regulate target sequences either negatively or positively, eliciting opposite actions to represent a Yin and Yang of RNA-mediated regulation. RNAa was originally accidentally discovered. In an attempt to silence transcription of the E-cadherin gene, Li et al. designed two dsRNAs targeting the E-cadherin gene promoter [1,2]. Since the E-cadherin promoter contains a CpG island preceding the transcription start site [3], the two shRNAs were designed to avoid regions with high GC content. To their surprise, transfection of both dsRNAs into cancer cells resulted in profound augmentation of E-cadherin mRNA and protein levels, rather than gene silencing [1,3]. The authors went on to demonstrate small RNA-mediated gene upregulation for the $p21^{WAF1/CIP1}$(p21) and vascular endothelial growth factor (VEGF) genes by targeting promoter regions of the corresponding genes, and named this phenomenon RNAa [1]. Small dsRNAs that serve as activators of gene expression have been defined as small activating RNAs (saRNAs) [1] or antigen RNAs (agRNAs) [4]. Soon after, Corey's group and others reported a great number of genes to display susceptibility to RNAa in a manner conserved in mammals, including humans, non-human primates, mice and rats [1,4–6].

In this review, we focus on the current advances of saRNA-mediated gene activation, particularly on the application of RNA transcriptional activation for cancer therapeutics and gene function study in cancer. We summarized the currently available literatures on the biology of RNAa and its underlying mechanisms, RNAa regulation of cell cycle and proliferation and the effect of RNAa on augmenting apoptosis and cellular senescence. We discussed what roles RNAa plays to overcome tumor invasion, metastasis and chemotherapy resistance. The application of RNAa in cancer therapeutics and its challenges were also addressed.

2. Mechanism and biology of RNAa

Although the phenomenon of RNAa has been identified for almost a decade, the application of this technology has not yet been as popular. The main reason for the limitation of its being widely used is the poor understanding of its mechanisms. Several excellent reviews by the original discoverers of RNAa have provided detailed description of possible mechanisms [7–9]. Based on current knowledge, the activation mechanism of RNAa involves both transcriptional and epigenetic alterations, and requires several enzymes (Fig. 1). In brief, saRNA is loaded onto an Argonaute (Ago) protein

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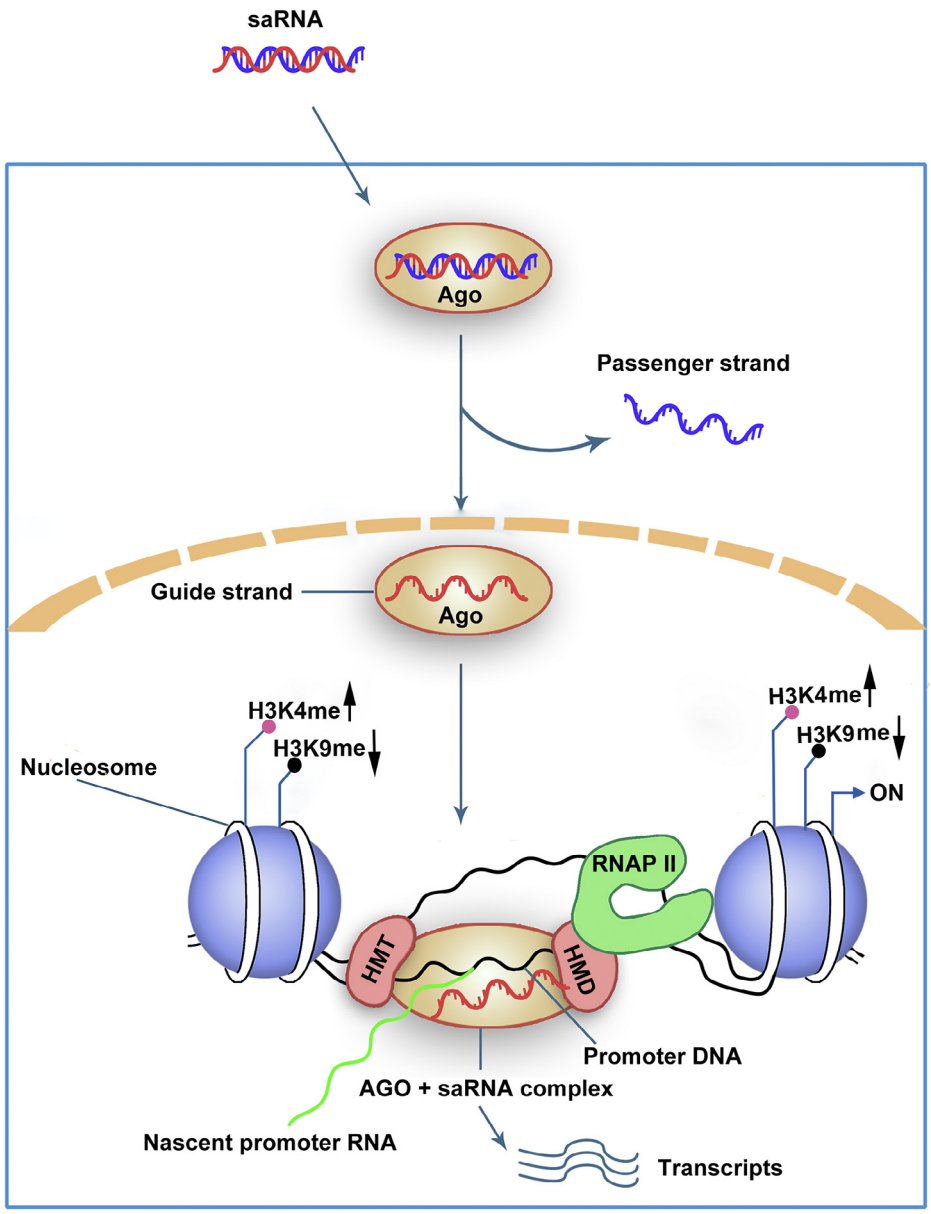

Fig. 1. A diagram describing saRNA-mediated transcription activation. The double-strand saran is loaded into an Ago family protein where the passenger strand (blue) is cleaved and discarded in cytoplasm, resulting in an active Ago-RNA complex with a guide strand (red). This complex moves through the nuclear membrane to target the gene promoter by the guide strand, which binds to a complementary sequence on the promoter DNA (black) or the nascent promoter transcript (green). This leads to the recruitment of a variety of histone remodeling enzymes, such as histone methyltransferases (HMTs) or demethylases (HDMs), causing removal of suppressive H3K9 me2 and H3K9 me3 (small black balls) or enrichment of active H3K4 me2 and H3K4 me3 (small pink balls). Consequently, the histone modification results in a more open chromatin structure for transcriptional activation.

which is usually in the cytoplasm. The loaded saRNAs are processed by removing one strand of saRNA to form an active Ago-RNA complex containing a selected guide strand RNA. The active complex is guided to its target regions by the guide strand, which may bind to complementary targets on either promoter DNA or nascent promoter RNA. Afterwards, histone modifying enzymes are recruited to the promoter to activate transcriptional expression through epigenetic modifications (Fig. 1).

Ago protein and histone modifying enzymes are required for RNAa action. In general, epigenetic modifications involve either the loss of suppressive epigenetic enzymes (i.e. histone H3 Lys9 dimethylation (H3K9me2), histone H3 lysine 9 trimethylation (H3K9me3), histone H3K9 acetylation (H3K9ac), and histone H3K4 acetylation (H3K4ac) or the gain of active epigenetic enzymes (i.e. H3K4 di-methylation (H3K4me2) and H3K4 tri-methylation (H3K4me3) [1,10,11]. Support of this suggestion comes from several studies including a recent one by Yang et al. They found that activation of prostate apoptosis response-4 (Par-4, also known as PAWR) is accompanied by reduction of H3K9me2 and increased H3K4me2 [12]. However, it is still out of the question that the epigenetic alterations are of causes or consequence. Although the dependence of RNAa on Ago proteins has been observed in most investigations [1], Janowski et al. did not find any Ago protein enriched in dsRNA target sites. In addition, other proteins, such as RNA Pol II and heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2/B1), have been reported to be associated with saRNA-directed activation of progesterone receptor (PR) and p21, respectively [6,13,14]. Interestingly, tissue specificity of the process has been reported [10], which suggests involvement of other cellular factors in the process. Moreover, whether other functional proteins are responsible for RNAa activity requires further dissection.