NRF2 signaling (Figure 1). Notably, the high efficacy of this drug was substantiated by using Taxotere-induced chemoresistant PCa cells, with which RG1603 induced morphological cell death. From the observation that the expression levels of reactive oxygen species (ROS) scavenger proteins were markedly reduced, it was clear that the elevated oxidative stress induced by RG1603 treatment mediated apoptotic cell death (Figure 1).

Lastly, the authors confirmed the high efficacy of RG1603 against prostate tumors in xenograft mouse models. Decreased expression of TERT, NRF2, and ROS scavenger and increased levels of an oxidative damage marker (8-OH-dG) were observed, which further validates the molecular mechanism of RG1603 leading to apoptotic cell death.

In sum, this work by Song and coworkers (Song et al., 2019) demonstrates that direct inhibition of *hTERT* gene expression via chaperone-like small molecules targeted to the specific loop motif of the repressive G4 assembly is a promising treatment option with desired efficacy and minimal side effects. Given that small molecules such as RG160 are structurally simple and relatively straightforward to synthesize in large quantities, this new G4-mediated gene-manipulating technology may develop into an almost ideal cancer therapy in the near future.

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RIBOTACs: Small Molecules Target RNA for Degradation

Sourav K. Dey[1] and Samie R. Jaffrey[1,*]
[1]Department of Pharmacology, Weill Cornell Medical College, Cornell University, New York, NY 10065, USA
*Correspondence: srj2003@med.cornell.edu
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Selective RNA degradation is a powerful strategy to combat diseases or study specific RNA function. In this issue of *Cell Chemical Biology*, Costales et al. (2019) develop a small molecule that mediates selective RNA degradation with the potential for cancer therapeutics.

Diverse classes of RNAs, including mRNAs, miRNAs, and long non-coding RNAs, are implicated in various diseases and are therefore potential drug targets (Cooper et al., 2009; Matsui and Corey, 2017). RNAs are traditionally targeted for degradation using antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs). Although some drugs in these classes are currently used in the clinic (e.g., Patisiran, Inotersen, Nusinersen, etc.), they still suffer from poor cellular uptake, low tissue-specific delivery (except liver and kidney), poor endosomal escape, and in some cases platform-specific toxicities (Setten et al., 2019). Therefore, it is important to develop new approaches that can target RNAs for degradation without the limitations of oligonucleotide therapies.

A promising new strategy to target RNAs for degradation is RIBOTACs (ribonuclease targeting chimeras). RIBOTACs are a new class of small molecules that have the potential to target diverse types of RNAs and may have certain advantages over oligonucleotide-based approaches. RIBOTACs are based on small molecules that selectively bind RNAs, especially RNAs that form intricate secondary and tertiary structures. They key innovation was converting RNA-binding molecules into RNA-degrading molecules. Disney, Costales, and colleagues initially developed the RIBOTACs concept by linking RNA-binding molecules to a small

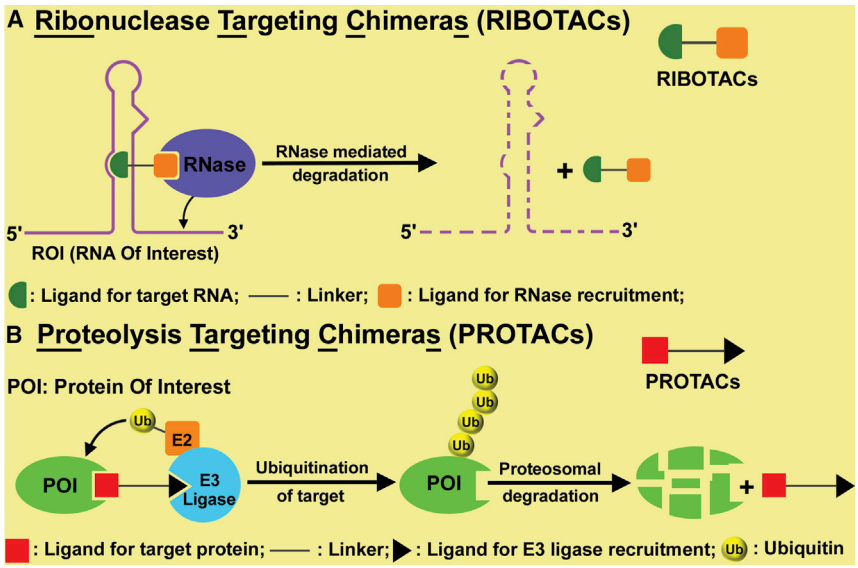

Figure 1. Comparison of RIBOTACs and PROTACs

(A) Ribonuclease targeting chimeras, or RIBOTACs, are bivalent molecules containing an RNA-binding module (green) and a ribonuclease (RNase) recruitment module (orange) joined by a linker (black line). Upon binding a target RNA, RIBOTACs recruit an RNase in close proximity of the target, thereby facilitating its degradation.

(B) Proteolysis targeting chimeras, or PROTACs, are also bivalent molecule where a protein binding ligand (red) is linked with an E3 ligase recruitment module (black) through a linker (black line). PROTACs position an E3 ligase in close proximity to a target protein leading to poly-ubiquitination of the target. This leads to degradation of the target protein by cellular proteasomal machinery.

molecule that binds and activates RNase L, an otherwise latent ribonuclease (Costales et al., 2018) (Figure 1A). The specific RNase L-recruiting component is a 2′-5′-linked tetra-adenylate (2′-5′ A_4). The tetra-adenylate is similar to endogenously produced oligoadenylates which are produced in cells during viral infection (Chakrabarti et al., 2011). The oligoadenylates activate the otherwise latent RNase L, causing it to dimerize and form an active ribonuclease. In this way, RIBOTACs recruit an active RNase L to an RNA of interest, causing its degradation (Figure 1A).

RIBOTACs are reminiscent of proteolysis targeting chimeras (PROTACS), which are also small molecules composed of two domains (Figure 1B), but in the case of PROTACS, the molecules induce an artificial association of target proteins with an E3 ligase, resulting in poly-ubiquitination of the target (Bondeson and Crews, 2017). This marks the target protein for proteasomal degradation (Figure 1B).

The initial demonstration of a RIBOTAC involved degradation of the precursor of microRNA 96 (pri-miR-96), which led to reduced levels of the mature microRNA (Costales et al., 2018). In the current paper, Costales et al. described TGP-210-RL, a RIBOTAC that mediates the selective degradation of pre-miR-210 (Costales et al., 2019). TGP-210-RL is derived from TGP-210, a small molecule that the Disney group previously developed that binds pre-miR-210 (Costales et al., 2017). TGP-210 was fused to the tetra-adenylate, thus forming the RIBOTAC.

Pre-miR-210 is an attractive therapeutic target because it is a precursor of microRNA miR-210, an essential microRNA for cancer survival in hypoxic niches. The authors confirmed the proposed mechanism of TGP-210-RL by showing that TGP-210-RL induces the formation of ternary complex comprising TGP-210-RL, the microRNA precursor, and RNase L. TGP-210-RL induced a marked reduction in miR-210 levels along with apoptosis in hypoxic cancer cells, demonstrating the general applicability of RIBOTACs for selective degradation of RNA and further demonstrating the ability of RIBOTACs to potentially target a RNA-based vulnerability in cancer cells (Costales et al., 2019).

RIBOTACs have advantages compared to oligonucleotide-based therapeutics. RIBOTACs may have superior pharmacokinetic properties over oligonucleotides because as a small molecule, they are likely to have access to a broad range of tissues when administered to animals. One major advantage of both PROTACs and RIBOTACs compared to conventional "occupancy-based" therapeutics are their catalytic nature (Figure 1). After a RIBOTAC binds and induces degradation of an RNA, it can bind to another RNA. In this way, low concentrations of the RIBOTAC can be used to achieve target RNA degradation.

The major drawback is that RIBOTACs are harder to develop than oligonucleotide-based therapeutics. In order to create a RIBOTAC, a researcher first needs to develop a small molecule that selectively binds the RNA of interest and not other RNAs in cells. The Disney group used Inforna, a platform for predicting RNA-binding small molecules, to develop the original TGP-210 (Costales et al., 2017). However, Inforna and other approaches for developing suitable RNA-binding ligands are much more difficult than designing an antisense oligonucleotide, which can simply be programmed using a complementary sequence to bind to a target RNA, with varying degrees of specificity.

Despite having certain favorable characteristics, TGP-210-RL showed only 2-fold increased activity compared to the parent molecule, which could be possibly due to slow cellular uptake. In its early days, one of the major challenges for PROTACs was the poor cellular uptake and low bioavailability that was caused by the high molecular weight of these chimeric molecules (Mullard, 2019). Similarly, the RIBOTACs molecule described in this paper are negatively charged and have a molecular weight > 2000. Indeed, the authors observed a 50% reduction in cellular uptake of TGP-210-RL compared to TGP-210 in cells. A further decrease might be expected in *in vivo* studies. Further iterations of RIBOTACs will likely involve more compact RNase-binding ligands. Interestingly, some small molecule activators of RNase L have been reported in the literature, although they show lower activity than the tetra-adenylate (Thakur et al., 2007). Nevertheless, the promising results shown in this study document the utility of this novel approach for targeting RNA and will stimulate efforts to develop RIBOTACs for other disease-relevant RNAs.

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