

Review

Cancer chemotherapy – ribonucleases to the rescue

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Abstract

Ribonucleases, once dismissed as uninteresting digestive enzymes, have been shown to have remarkable biological activities. Onconase®, from the Northern leopard frog, is currently in clinical trials as a cancer chemotherapeutic. Recent research has revealed some key factors responsible for the cytotoxicity of

ribonucleases, and may lead to a new class of drugs. © 2001 Published by Elsevier Science Ltd.

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1. Introduction

Ribonucleases are best known for their ability to cleave RNA. Yet, a growing number of these enzymes are being shown to have unusual biological activities. Bovine pancreatic ribonuclease (or RNase A; EC 3.1.27.5) was studied extensively during the 1960s and 1970s as a model system, in part because of its prevalence in an accessible source (the cow pancreas) and also because of its ease of purification and small size (14 kDa). RNase A was the first enzyme to have its sequence determined and the third to have its structure revealed. RNase A was also used in many early protein folding studies. Indeed, the 1972 Nobel Prize for Chemistry was awarded jointly to Stanford Moore, William Stein, and Christian Anfinsen for their collective work on RNase A. In 1984, Bruce Merrifield was awarded the Nobel Prize for developing chemical synthesis on a solid matrix, and he likewise used RNase A as a model.

RNase A is but the best known member of a superfamily of secretory enzymes that operate at the crossroads of transcription and translation by catalyzing RNA degradation (Fig. 1) [1,2]. Interest in ribonucleases was renewed after some of these proteins were shown to be much more than digestive enzymes [3,4]. For example, angiogenin is a

plasma enzyme that promotes the growth of new blood vessels [5], and bovine seminal ribonuclease, a unique dimeric homolog of RNase A, has immunosuppressive, embryotoxic, aspermatogenic and antitumor activities [6]. Onconase, an amphibian protein, is toxic to tumor cells both in vitro and in vivo [7].

The unexpected biological activities of ribonucleases present novel opportunities for treating disease. Onconase, for example, is in phase III human clinical trials as a cancer chemotherapeutic, and new research is revealing the basis of its specific toxicity for cancer cells. These revelations are inspiring means to endow mammalian ribonucleases with specific and useful cytotoxicity [8].

2. Pancreatic-type ribonucleases

RNase A is secreted in large quantities by the bovine pancreas, presumably to digest the large amount of RNA produced by microbial residents of the rumen [9]. The in vivo function of RNase A is enhanced by two exceptional features of the enzyme – high catalytic activity and high conformational stability.

RNase A catalyzes the cleavage of the P–O^{5'} bond of RNA on the 3' side of pyrimidine nucleosides (Fig. 2) [2,8] in one of the most efficient catalytic processes identified to date ($k_{\text{cat}}/K_{\text{m}} > 10^9 \text{ M}^{-1} \text{ s}^{-1}$ [10]). The structure of RNase A, which resembles a kidney, is stabilized by four disulfide bonds that involve all eight of its cysteine residues (Fig. 3b) [11,12]. RNase A has a T_{m} (which is the temperature

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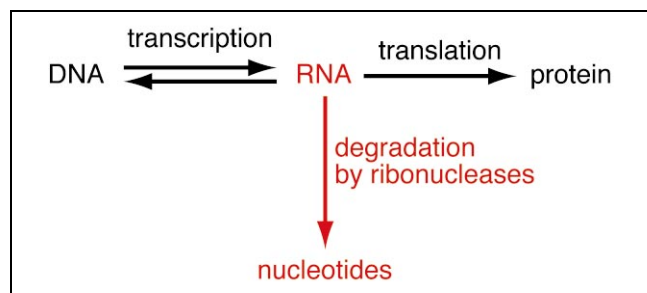


Fig. 1. Biochemical basis for the potential cytotoxicity of ribonucleases.

at the midpoint of thermal denaturation) of 62°C [13], and its denaturation is fully reversible [14].

Ribonucleases can be cytotoxic because cleavage of RNA renders indecipherable its encoded information (Fig. 1). The cytotoxicity of pancreatic-type ribonucleases was discovered in the 1950s. In these experiments, RNase A was shown to be toxic to tumor cells, both in vitro [15] and in vivo [16,17]. Although effects were observed only after milligrams of enzyme were injected into solid tumors, these early studies were the first to demonstrate the potential of pancreatic-type ribonucleases as cancer chemotherapeutics.

3. Ribonuclease inhibitor

Remarkably, the most potent known inhibitor of RNase A, which is secreted from cells, is a *cytosolic* protein. Ribonuclease inhibitor (or RI; 50 kDa) constitutes 0.01–0.1% of the total protein in the cytosol of mammalian cells [18,19]. Homologous RI proteins from porcine (pRI) and human (hRI) have been described in detail. Both of these RIs contain 15 sequential leucine-rich β - α repeats arranged in the shape of a horseshoe [20]. In addition, pRI and hRI contain 30 and 32 reduced cysteine residues, respectively. RI requires a reducing environment (such as the cytosol) to maintain its activity. Oxidation of a single cysteine residue quickly leads to the oxidation of the remaining cysteine residues. Once oxidized, RI loses its ability to bind to RNase A and is degraded rapidly by cellular proteases [21].

RI forms a 1:1, noncovalent complex with RNase A (Fig. 4). The K_d value of the pRI·RNase A complex is 6.7×10^{-14} M [22], and that of the hRI·RNase A complex is 4.4×10^{-14} M [23]. (These values are likely to be even smaller in the cytosol [24].) Seven of the 12 RNase A residues that contribute most to substrate binding or turnover participate in intermolecular contacts with the inhibitor [25,26]. The four disulfide bonds of RNase A and the 30 reduced cysteine residues of pRI are preserved in the complex. pRI and hRI are potent inhibitors of other mammalian pancreatic-type ribonucleases, including angiogenin and human pancreatic ribonuclease [23,27,28]. The action of RI is, however, class-specific – RIs from amphib-

ia and birds are ineffective inhibitors of mammalian pancreatic-type ribonucleases [29].

The biological role of RI has yet to be defined rigorously [19]. Because all known RI ligands are secreted ribonucleases, it seems likely that RI serves to protect cellular RNA should a secretory ribonuclease inadvertently enter the cytosol. In addition, RI may regulate other biological actions of ribonucleases. Regardless of its function, the pronounced sensitivity of RI to oxidation implies a possible mechanism for the regulation of its inhibitory activity.

4. Onconase

Onconase is a homolog of RNase A present in the oocytes and early embryos of *Rana pipiens*, the Northern leopard frog [7,30]. Discovered on the basis of its antitumor activity, onconase was immediately recognized as a promising cancer chemotherapeutic agent [31]. That onconase was a ribonuclease was only discovered after its sequence became known [32].

The amino acid sequence of onconase is ~30% identical to that of RNase A (Fig. 3a) [32]. The tertiary structure of onconase is also similar to that of RNase A (Fig. 3b) [33]. Onconase is crosslinked by four disulfide bonds, three of which are conserved in RNase A (Fig. 3b). The T_m of onconase is 90°C, nearly 30°C higher than that of RNase A [34], a difference that is largely due to its synapomorphic carboxy-terminal disulfide bond [35]. Removing this disulfide bond not only reduces the thermal stability of onconase to that of RNase A, but also compromises its cytotoxic activity. The amino-terminus of onconase is blocked by a pyroglutamyl residue, which is formed by the cyclization of an amino-terminal glutamine residue.

Like RNase A, onconase catalyzes cleavage of the P–O^{5'} bond of RNA on the 3' side of pyrimidine nucleosides. The principal catalytic residues of RNase A are conserved in onconase (Fig. 3a), implying that RNase A and onconase catalyze RNA cleavage using similar mechanisms. Despite this similarity, the catalytic efficacy of onconase is much lower than that of RNase A [27,32,34,35]. Interestingly, RI is not a potent inhibitor of onconase catalysis. The K_i value for the inhibition of onconase by RI is estimated to be $\geq 10^{-6}$ M, which is more than 10^7 -fold greater than the K_d value for the RI·RNase A complex [27].

Onconase is a cytostatic agent that arrests the cell cycle in G1 phase [31,36], as well as a cytotoxin [37]. The LD₅₀ value for onconase cytotoxicity in vitro depends on the type of cancer cell, but is frequently near 10^{-7} M. Proliferating cells are more susceptible to onconase than are quiescent cells [38]. Correspondingly, agents that increase the rate of cell proliferation potentiate onconase cytotoxicity, and agents that decrease the rate of cell proliferation reduce onconase cytotoxicity. LD₅₀ values are even lower when onconase is administered in combination with small-

molecule chemotherapeutic agents, including tamoxifen, trifluoperazine, cisplatin, lovastatin, and vincristine [39–41]. In addition to its anti-cancer activity, onconase has intriguing anti-viral properties. At 10^{-8} M, onconase inhibits HIV-1 replication in chronically infected human cells by degrading viral RNA [42,43]. Significantly, a 10^{-8} M dose of onconase does not kill the infected cell.

Onconase is an effective chemotherapeutic agent in animals. In one study, tumor cells were injected into the intraperitoneal cavity of mice, and treatment with onconase was initiated 24 h later [44]. All treatment schedules increased significantly the median time to death, compared to that of untreated animals (18 days). Weekly injections of 40 μ g/mouse yielded the greatest number of long-term survivors. In this test group, six of 18 mice survived for >220 days with no evidence for onset of disease. The most consistent side effect of onconase was weight loss, which increased in severity with the frequency and size of the dose.

Onconase has been tested in phase I and phase II human clinical trials for treatment of numerous solid tumors, including lung and pancreatic cancers [45,46]. In these trials, onconase appeared to have a favorable impact on the median survival time of the patients. Renal toxicity is dose-limiting, but is reversible upon discontinuation of treatment. More recently onconase has been tested, in combination with tamoxifen, in a phase III trial for treatment of patients with advanced pancreatic adenocarcinoma. This trial was discontinued in 1998 because tolerated levels of onconase did not offer a significant therapeutic advantage compared to 2'-deoxy-2',2'-difluorocytidine (Gemzar®). A phase III trial for treatment of malignant mesothelioma, an asbestos-related lung cancer, is still ongoing.

5. *Rana catesbeiana* and *Rana japonica* ribonucleases

Ribonucleases with chemical and biological properties remarkably similar to those of onconase have also been isolated from the oocytes of *Rana catesbeiana* (bullfrog) and *Rana japonica* (Japanese rice paddy frog) [7,30]. The *R. catesbeiana* and *R. japonica* ribonucleases share ~50% amino acid sequence identity with onconase (Fig. 3a)

[47–49]. The structure of the *R. catesbeiana* ribonuclease in solution resembles the crystalline structure of onconase (Fig. 3b). Each of the four disulfide bonds in the *R. catesbeiana* ribonuclease has a counterpart in onconase. Also similar to onconase, *R. catesbeiana* ribonuclease has unusually high conformational stability – its T_m is >75°C. The *R. japonica* ribonuclease, which has an unknown structure, retains the cysteine residues and stability of onconase (Fig. 3b). The *R. catesbeiana* and *R. japonica* ribonucleases catalyze cleavage of the P–O^{5'} bond of RNA 3' to pyrimidine nucleosides. Significantly, the ribonucleolytic activity of the *R. catesbeiana* ribonuclease is not inhibited by hRI [50]. The effect of hRI on the *R. japonica* ribonuclease is unknown.

The *R. catesbeiana* and the *R. japonica* ribonucleases are cytotoxic to various cancer cells in vitro with LD₅₀ values of 10^{-7} – 10^{-6} M [51,52], and the *R. catesbeiana* ribonuclease has been shown to be an effective chemotherapeutic agent in vivo [50]. In this study, tumor cells were injected into the intraperitoneal cavity of mice, followed by daily i.p. injections of the *R. catesbeiana* ribonuclease. The survival times of treated animals were significantly higher than those of control animals. As with onconase, treatment with the *R. catesbeiana* ribonuclease caused weight loss in the test animals [50].

6. The structure–function problem

The cytotoxicity of onconase is dependent on its ribonucleolytic activity [27,37,53,54], as is the cytotoxicity of the *R. catesbeiana* ribonuclease [55]. Although the substrate specificity of the ribonucleolytic activity of onconase varies from assay to assay (rRNA is degraded in cultured cells treated with onconase [37] and tRNA is degraded preferentially in a reticulocyte lysate [27,56]), it is clear that cytotoxic ribonucleases cause cell death by degrading RNA. Even though RNase A has greater ribonucleolytic activity than does onconase or the *R. catesbeiana* ribonuclease, RNase A is far less cytotoxic.

Does RI play an integral role in ribonuclease cytotoxicity? If so, one would expect a variant of RNase A that is resistant to inactivation by RI to be cytotoxic. The structure of the pRI·RNase A complex suggests which residues

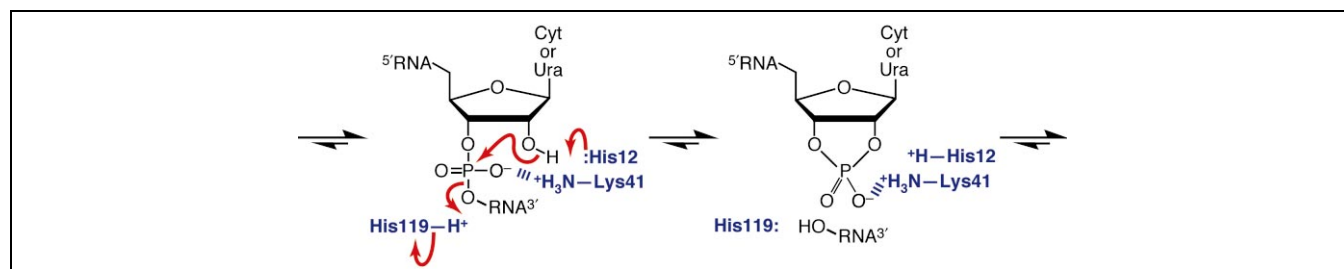


Fig. 2. Putative mechanism for catalysis of RNA cleavage by RNase A. His12, Lys41 and His119 are conserved in all homologous ribonucleases (see Fig. 3a).

a

a

Ribonuclease A	1	K	E	T	A	A	K	F	E	R	Q	H	M	D	S	S	T	S	A	A	S	S	S	N	Y	C	N	Q	M	M	-	K	S	R	N	L	T	K	D	R	C	K	P	
Onconase		-	-	<	E	D	W	L	T	F	Q	K	K	H	I	T	N	T	-	-	-	P	I	I	N	-	C	D	N	I	M	S	T	N	L	F	-	-	-	H	C	K	D	
<i>Rana catesbeiana</i>		-	-	<	E	N	W	A	T	F	Q	Q	K	H	I	I	N	T	-	-	-	S	S	P	N	-	C	N	T	I	M	D	N	N	I	Y	I	V	G	G	Q	C	K	R
<i>Rana japonica</i>		-	-	<	E	N	W	A	K	F	Q	E	K	H	I	P	N	T	-	-	-	S	N	I	N	-	C	N	T	I	M	D	K	S	I	Y	I	V	G	G	Q	C	K	E

Ribonuclease A	50	V	N	T	F	V	H	E	S	L	A	D	V	Q	A	V	C	S	Q	K	N	V	A	C	K	N	G	Q	T	N	C	Y	Q	S	Y	S	T	M	S	I	T	D	C	R	E
Onconase		K	N	T	F	I	Y	S	R	P	E	P	V	K	A	I	C	K	G	I	I	A	S	K	N	V	L	T	T	S	E	F	Y	-	-	-	-	-	L	S	D	C	-	-	
<i>Rana catesbeiana</i>		V	N	T	F	I	I	S	S	A	T	T	V	K	A	I	C	T	G	V	-	I	N	M	N	V	L	S	T	T	R	F	Q	-	-	-	-	-	L	N	T	C	T	R	
<i>Rana japonica</i>		R	N	T	F	I	I	S	S	A	T	T	V	K	A	I	C	S	G	A	S	T	N	R	N	V	L	S	T	T	R	F	Q	-	-	-	-	-	L	N	T	C	I	R	

Ribonuclease A	90	T	G	S	S	K	Y	P	N	C	A	Y	K	T	T	Q	A	N	K	H	I	V	A	C	E	G	N	P	Y	V	P	V	H	F	D	A	S	V	-	-	-		
Onconase		-	N	V	T	S	R	P	-	C	K	Y	K	L	K	K	S	T	N	K	F	C	V	T	C	E	N	Q	-	-	A	P	V	H	F	-	V	G	V	G	S	C	-
<i>Rana catesbeiana</i>		T	S	I	T	P	R	P	-	C	P	Y	S	S	R	T	E	T	N	Y	I	C	V	K	C	E	N	Q	-	-	Y	P	V	H	F	-	A	G	I	G	R	C	P
<i>Rana japonica</i>		S	A	T	A	P	R	P	-	C	P	Y	N	S	R	T	E	T	N	V	I	C	V	K	C	E	N	R	-	-	L	P	V	H	F	-	A	G	I	G	R	C	-

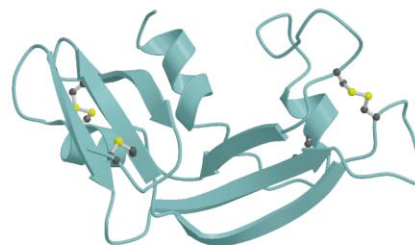
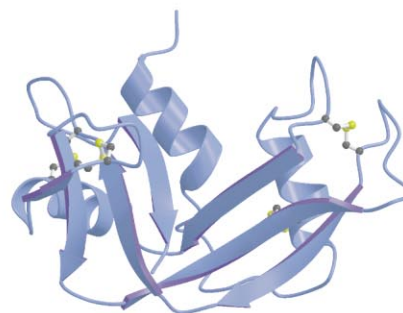
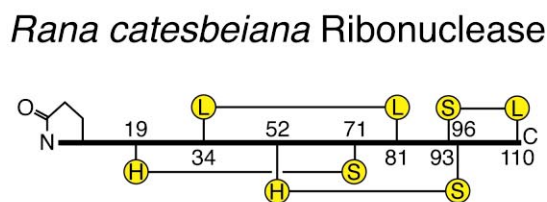
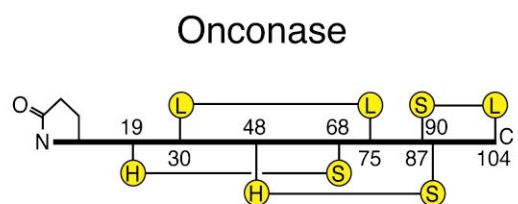
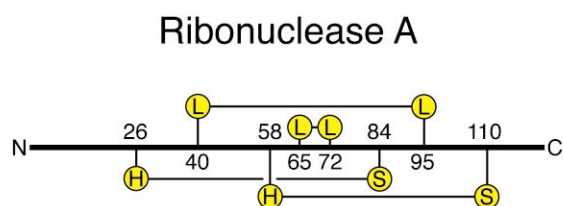
b

Fig. 3. (a) Amino acid sequences of RNase A and three of its cytotoxic homologs. Sequences were aligned using the PILEUP program (Genetics Computer Group, Version 10; Madison, WI, USA) with a gap creation penalty of 8 and a gap extension penalty of 2. Residues are numbered according to RNase A. RNase A residues that contact porcine ribonuclease inhibitor in the pRI-RNase A complex are white on black. Conserved residues are boxed. The three residues most important for catalysis by RNase A (His12, Lys41 and His119), and the corresponding residues in the cytotoxic homologs, are blue. Cysteine residues are yellow. (b) Three-dimensional structures of RNase A, onconase, and the *Rana catesbeiana* ribonuclease. The secondary structural context of each half-cystine is indicated by H (helix), S (sheet), or L (loop). Ribbon diagrams were created with the programs MOLSCRIPT [76] and Raster3d [77] using coordinates derived from X-ray diffraction [12,33] or NMR spectroscopy [78].

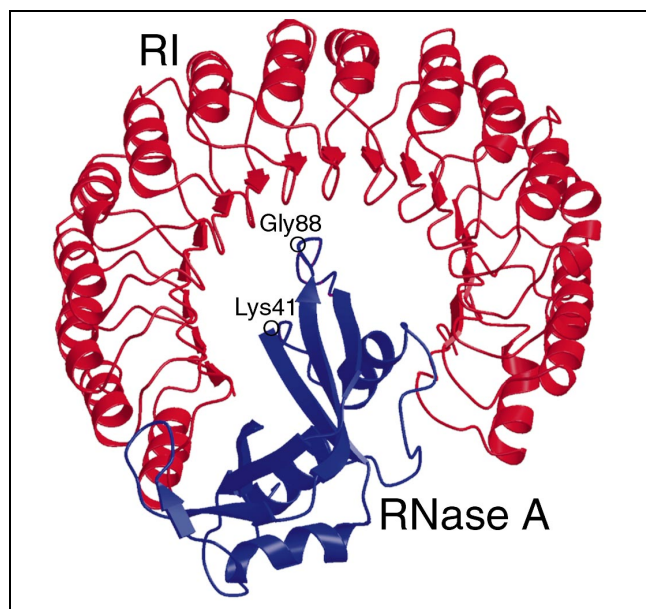


Fig. 4. Three-dimensional structure of the complex between porcine ribonuclease inhibitor (red) and RNase A (blue). Ribbon diagrams were created with the programs MOLSCRIPT [76] and Raster3d [77] using coordinates derived from X-ray diffraction [25].

are essential for the interaction of these two proteins. Replacing Gly88, which lies in a hydrophobic pocket defined by three tryptophan residues of pRI (Fig. 4) [25], is one option [34]. The Gly88 → Arg (G88R) substitution causes steric and electrostatic strain in the enzyme-inhibitor complex, decreasing the susceptibility of RNase A to inactivation by RI. The K_i value for the G88R RNase A is 10^4 -fold greater than that for RNase A. Moreover, the conformational stability and catalytic activity of the variant RNase A are comparable to those of the wild-type RNase A. Most significantly, the G88R RNase A is toxic to a human leukemia cell line (LD_{50} 7 μ M) [34]. By comparison, the LD_{50} value for onconase is 0.5 μ M, and a 50 μ M dose of wild-type RNase A has no effect on tumor cell viability. It is clear that wild-type RNase A has all of the properties necessary to be a potent cytotoxin, except for resistance to RI.

Because the physico-chemical properties of RNase A are well defined, the G88R RNase A provides a unique opportunity to dissect the contributions of catalysis and conformational stability to ribonuclease cytotoxicity. Several RNase A variants that include the G88R substitution along with a second substitution in the enzymic active site have been generated [57,58]. The side chain of Lys41 donates a hydrogen bond to the transition state during RNA hydrolysis, and also interacts with pRI residues Tyr430 and Asp431 in the pRI-RNase A complex (Figs. 2 and 4). The double variant Lys41 → Arg (K41R)/G88R RNase A is 20-fold less susceptible to inhibition by hRI and is threefold more cytotoxic than G88R RNase A [57]. This result is striking because the K41R substitution decreases ribonucleolytic activity by 10^2 -fold. Thus, the increase in K_i seems to compensate for diminished catalytic activity.

The four disulfide bonds in RNase A make a substantial contribution to the conformational stability of the enzyme (Fig. 3b). Cytotoxins with a range of T_m s can be created by adding a fifth disulfide bond to G88R RNase A, or by removing one of the native disulfide bonds from G88R RNase A [58]. Interestingly, as the conformational stability of a variant increases, so does its cytotoxicity. What is the basis for this correlation? As the conformational stability of an RNase A variant increases, the enzyme becomes less susceptible to proteolytic degradation [58]. The cytotoxicity of a ribonuclease appears, therefore, to be dependent on avoiding intracellular proteolysis.

7. Cellular routing of cytotoxic ribonucleases

As cytotoxins, ribonucleases are administered extracellularly. To kill cancer cells, however, cytotoxic ribonucleases hydrolyze RNA – a cytosolic molecule. At present, it is unclear how this extracellular protein reaches the cytosol. Limited, but intriguing preliminary data do, however, suggest a route.

The first step in ribonuclease cytotoxicity is an interaction between the ribonuclease and the plasma membrane

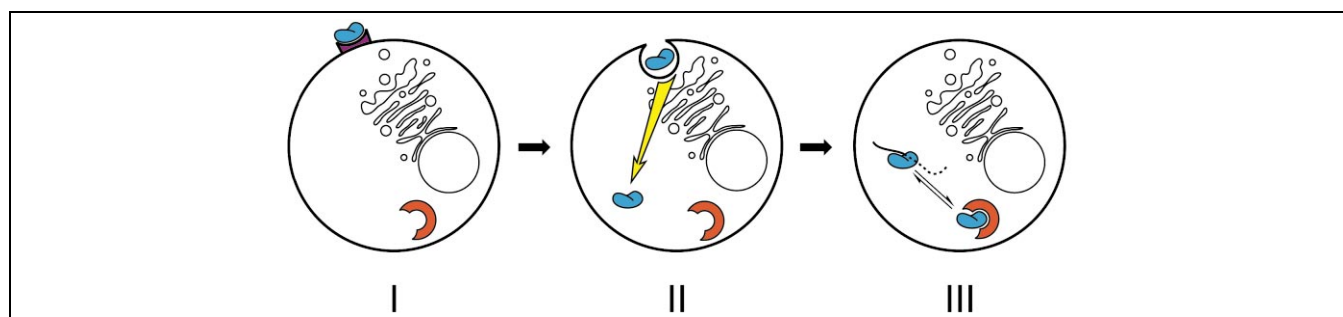


Fig. 5. Putative cellular routing of cytotoxic pancreatic-type ribonucleases. (I) The ribonuclease first interacts with the surface of the target cell. Onconase and the *R. catesbeiana* and *R. japonica* ribonucleases appear to bind receptors on the plasma membrane. (II) The ribonuclease is internalized by endocytosis and crosses a lipid bilayer to reach the cytosol. The mechanism of bilayer transversal is unknown. (III) In the cytosol, ribonucleases encounter the RI protein. Ribonucleases that evade RI catalyze cleavage of cellular RNA, which leads to cell death.

of the target cell (Fig. 5). Onconase binds to specific sites on the plasma membrane of cultured glioma cells with K_d values of 6.2×10^{-8} M and 2.5×10^{-7} M [37]. Receptors for this interaction have not been identified. It is also unclear if these receptors are intrinsic to the glioma cells, or if onconase binds to other cancer cells using the same interactions. The cytotoxic ribonucleases from *R. catesbeiana* and *R. japonica* also bind specifically to the plasma membrane of cancer cells. Indeed, these enzymes were first identified as sialic acid binding lectins that agglutinate cancer cells specifically [59–61]. This agglutination is inhibited by sialoglycoproteins and the ganglioside fraction of human erythrocyte membranes, but is not blocked by monomeric sialic acid [59,60]. Agglutination is also inhibited by nucleotides, indicating that the active site residues contribute to binding [61]. Finally, pretreatment with sialidase protects tumor cells from both agglutination and cytotoxicity by the *R. catesbeiana* ribonuclease [51,60]. It is likely, therefore, that the same receptor renders cancer cells susceptible to agglutination and cytotoxicity by the *R. catesbeiana* and *R. japonica* ribonucleases. Surprisingly, onconase has been reported not to cause tumor cell agglutination [32].

Many research groups have created fusion proteins to enhance the interactions between ribonucleases and the plasma membrane [62,63]. The LD_{50} values for the cytotoxicity of such targeted ribonucleases are close to nanomolar. These fusion proteins are markedly less toxic to cells that do not express receptors for the targeting epitope. RI susceptibility has not been quantitated for most of these proteins. In one notable exception [64], transferrin was coupled to residue 89 of human pancreatic ribonuclease. Analogous to the G88R substitution in RNase A, transferrin sterically prevents RI from binding. In addition, transferrin targets the conjugate to the surface of cells expressing the transferrin receptor. In the presence of retinoic acid (a small molecule that disrupts the Golgi apparatus), the ribonuclease–transferrin conjugate is toxic to cancer cells with an LD_{50} value of 2 nM. Some ribonuclease fusion proteins could be cytotoxic even if they are inhibited by RI [65]. The targeting domain of a fusion protein enables receptor-mediated entry into the cell, which could lead to the accumulation of ribonucleolytic activity in the cytosol. There, the fusion protein could titrate RI, ultimately leaving ribonucleolytic activity unchecked.

After binding to a plasma membrane receptor, onconase must gain access to RNA (Fig. 5). Small molecules that inhibit ATP synthesis also abolish onconase cytotoxicity [37]. This result is consistent with onconase entering the cell by endocytosis, an energy-dependent process. Like retinoic acid, monensin is a small molecule that disrupts intracellular trafficking. Both retinoic acid and monensin potentiate onconase cytotoxicity [53], indicating that onconase is able to reach the cytosol more efficiently in cells

with damaged vesicles. Retinoic acid may, however, alter the intracellular routing of onconase. Brefeldin A stops both forward and retrograde vesicular transport between the endoplasmic reticulum and the Golgi apparatus. Brefeldin A blocks onconase cytotoxicity in the presence, but not the absence, of retinoic acid [53].

Cytotoxic ribonucleases face a topological problem – regardless of their path into the cell, they must cross a lipid bilayer to reach the cytosol. The location and mechanism of bilayer transversal is unknown. Unlike many bacterial toxins, which have distinct catalytic and translocation domains [66,67], RNase A and its homologs have but a single domain (Fig. 3b). Moreover, ribonucleases are highly cationic, not lipophilic. In addition, their multiple interweaving disulfide bonds impose girth. These disulfide bonds must remain intact during translocation, as the reducing environment of the cytosol would not permit re-oxidation. (The disulfide bonds of native RNase A are virtually inaccessible to solvent [13] and have considerable kinetic stability in a highly reducing environment [68].) It appears that only a few molecules of a cytotoxic ribonuclease are required to kill a cell [69]. Hence, delineating the transbilayer movement of ribonucleases in molecular terms is a formidable challenge [70].

8. Basis for therapeutic index

In cell culture systems, animal models, and human clinical trials, the *Rana* ribonucleases are more toxic to cancer cells than to noncancer cells. The basis for this favorable therapeutic index is unknown. Changes to the plasma membrane – perhaps upregulation of a receptor – may increase the susceptibility of cancer cells to cytotoxic ribonucleases. Indeed, the density of sialic acid-rich gangliosides is elevated in some types of cancers [71,72]. Alternatively, the cellular routing of ribonucleases or cytosolic RI levels could differ between cancer cells and their normal counterparts. Finally, the rapid proliferation of cancer cells could make them more reliant on the integrity of their RNA (Fig. 1).

A chemotherapeutic agent based on a human protein is likely to be preferable to one based on an amphibian protein. The *Rana* ribonucleases have several undesirable side effects (vide supra). Onconase is identical to its human homolog in only 31 of its 104 residues. When ribonucleases are injected into mice 50% of onconase is found in the kidney after 3 h, compared with only 1% of human pancreatic ribonuclease [73,74]. Renal retention could, therefore, limit the efficacy of onconase, as it limits dosage [45,46]. Already, a dimeric variant of human pancreatic ribonuclease, which evades hRI, has been shown to be toxic to cancer cells in vitro [75]. Indeed, the creation and testing of potent cytotoxic variants of human pancreatic ribonuclease is a most exciting development.

9. Prospects

Recent work on cytotoxic ribonucleases is providing a framework for the development of a new class of cancer chemotherapeutic agents. Moreover, efforts to reveal the mechanism of ribonuclease cytotoxicity are revealing new questions for chemical biologists. Why are cancer cells especially vulnerable to pancreatic-type ribonucleases? How does a highly charged protein cross a lipid bilayer? Would a small-molecule antagonist of RI potentiate the cytotoxicity of exogenous or endogenous ribonucleases? The plethora of basic research carried out on RNase A during the 20th century is paying off now in a most unexpected way.

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