Published in final edited form as:

Eur J Pharmacol. 2009 December 25; 625(1-3): 181–189. doi:10.1016/j.ejphar.2009.06.067.

Ribonucleases as potential modalities in anticancer therapy

Wojciech Ardelt, Barbara Ardelt, and Zbigniew Darzynkiewicz

Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, New York USA

Abstract

Antitumor ribonucleases are small (10–28 kDa) basic proteins. They were found among members of both, ribonuclease A and T1 superfamilies. Their cytotoxic properties are conferred by enzymatic activity, i.e., the ability to catalyze cleavages of phosphodiester bonds in RNA. They bind to negatively charged cell membrane, enter cells by endocytosis and translocate to cytosol where they evade mammalian protein ribonuclease inhibitor and degrade RNA. Here, we discuss structures, functions and mechanisms of antitumor activity of several cytotoxic ribonucleases with particular emphasis to the amphibian Onconase, the only enzyme of this class that reached clinical trials. Onconase is the smallest, very stable, less catalytically efficient and more cytotoxic than most RNase A homologues. Its cytostatic, cytotoxic and anticancer effects were extensively studied. It targets tRNA, rRNA, mRNA as well as the non-coding RNA (microRNAs). Numerous cancer lines are sensitive to Onconase; their treatment with 10 – 100 nM enzyme leads to suppression of cell cycle progression, predominantly through G₁, followed by apoptosis or cell senescence. Onconase also has anticancer properties in animal models. Many effects of this enzyme are consistent with the microRNAs, one of its critical targets. Onconase sensitizes cells to a variety of anticancer modalities and this property is of particular interest, suggesting its application as an adjunct to chemotherapy or radiotherapy in treatment of different tumors. Cytotoxic RNases as exemplified by Onconase represent a new class of antitumor agents, with an entirely different mechanism of action than the drugs currently used in the clinic. Further studies on animal models including human tumors grafted on severe combined immunodeficient (SCID) mice and clinical trials are needed to explore clinical potential of cytotoxic RNases.

Index words

ribonucleases; onconase; amphinase; structure/function; cancer; apoptosis

1. Introduction

Ribonucleases (RNases) are potentially cytotoxic by virtue of their ability to degrade RNA (Arnold, 2008; Matousek et al., 2003) and therefore to inhibit protein biosynthesis at both, transcription and translation stages. However, most known RNases do not exhibit innate cytotoxic or cytostatic activities.

Corresponding author: Dr. W. Ardelt, Brander Cancer Research Institute at NYMC, Department of Pathology, BSB 438, Valhalla, NY 10595, Tel: 914-594-3780, Fax: 914-594-3790, bw_ardelt@hotmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Bovine pancreatic ribonuclease A (RNase A), EC 3.1.27.5 (reviewed by Raines, 1998) was the first enzyme of this group tested for a possible anti-cancer activity *in vitro* (Ledoux, 1956; Ledoux, and Baltus, 1954; Ledoux and Revell, 1955) and *in vivo* (Ledoux, 1955a, 1955b; Aleksandrowicz et al., 1958; Telford et al., 1959). The results were contradicting; some authors did not observe any effects others reported anti-cancer activity only when high amounts of crystalline enzyme were employed e.g., milligrams injected to solid tumors.

The first clinical study of RNase A as a potential anti-cancer drug was performed by Korean researchers (Yun et al., 1972). Again, in most of the 23 patients no therapeutic effects were observed. In some cases, however, especially when the enzyme was applied directly to the tumors in large amounts (up to 3 mg per kg of body weight/day), a regression or even disappearance of cancer tissue occurred. Despite some encouraging observations the interest in a therapeutic potential of RNases faded for some time but it was revived later when RNases cytotoxic to cancer cells at much lower concentrations, were discovered.

Enzymes with an innate anti-cancer activity were found within both, RNase A and RNase T1 super-families. Among the members of the former, the most known are bovine seminal plasma RNase and Onconase (the latter is the only RNase so far that reached clinical trials) as well as some other enzymes of amphibian origin. Binase and some ribotoxins are representatives of the latter superfamily of microbial RNases.

In this article we review structures and functions as well as mechanisms of antitumor activity and therapeutic potential of some natural RNases with intrinsic cytotoxic activity to cancer cells.

2. Bovine Seminal Ribonuclease

This enzyme (BS-RNase) was independently discovered by Hosokawa and Irie (1971), D'Alessio et al. (1972a) and Dostal and Matousek (1972). It is the only known RNase with quaternary structure. Its molecule is a natural dimer that consist of two identical subunits linked by two disulfide bonds and some non-covalent interactions (D'Alesio et al., 1972b, 1991). Amino acid sequence of the BS-RNase subunit (Suzuki et al., 1987) and its crystallographic structure (Capasso et al., 1983; Mazzarella, et al., 1993) clearly demonstrated that this enzyme belongs to the superfamily of pancreatic RNase A (reviewed by Beintema et al., 1988; Dyer and Rosenberg, 2006).

Like RNase A, the single chain of BS-RNase subunit consists of 124 amino acid residues in a sequence over 80% identical to that of the former enzyme. Catalytic residues, His12, Lys41 and His119 as well as the four disulfide bridges of RNase A are strictly conserved in BS-RNase. The most pronounced difference in the amino acid sequences of these enzymes is the presence of two consecutive Cys residues at positions 31 and 32 of the BS-RNase subunit. Those residues form two inter-chain disulfide bonds, Cys31 of one subunit with Cys 32 of the other, what results in the enzyme dimerization (Di Donato and D'Alessio, 1973). Dimeric enzyme (molecular weight 27218 and isoelectric point 10.3) is a mixture of two distinct quaternary forms denoted M=M and MxM (Piccoli et al., 1992). In the former, the two subunits are held together merely by the two disulfide bridges while in the latter the subunits interact also by swapping their N-terminal α -helices. More discussion of the structure and function of this interesting enzyme can be found in the comprehensive review by D'Alessio et al. (1997).

Enzymatic activity is critical for BS-RNase cytotoxicity and, therefore, for the anticancer activity and other biological effects of this enzyme (Kim et al., 1995a). However, as demonstrated by Vestia et al. (1980) and Kim et al. (1995b), a single chain subunit (obtained by selective reduction of inter-chain disulfides) that is even more catalytically active than the original dimeric BS-RNase, is not cytotoxic. Also, as shown by Murthy and Sirdesmukh, 1992,

the subunit is strongly inhibited by mammalian RNase inhibitor that does not interact with the natural dimeric enzyme. Based on those and other observations, Kim et al. (1995c) proposed the following general mechanism of BS-RNase cytotoxicity. The enzyme as a mixture of both M=M and MxM forms in equilibrium enters the cell by absorptive endocytosis. In the reducing environment of cytosol the M=M form is reduced to two monomeric subunits which are inactivated by RI. The MxM form stabilized by the noncovalent interactions (domain swapping) stays dimeric, evades ribonuclease inhibitor and degrades intracellular RNA, especially rRNA, which leads to cytotoxicity.

RNase inhibitor is a 50 kDa protein present in mammalian cell cytosol (reviewed by Hofstenge, 1997; Shapiro, 2001; Dickson et al., 2005) able to form extremely tight complexes with most of mammalian RNases, and this way, to abolish their catalytic activities. As we discuss later, other cytotoxic RNases are also resistant to this inhibitor. Thus, it seems that the insensitivity to mammalian RNase inhibitor may be a prerequisite for RNase cytotoxicity (for recent review see Rutkoski and Raines, 2008).

3. Onconase and Amphinase from *Rana pipiens*

In late 1980s Alfacell Corporation studied an extract of early embryos of leopard frog (*Rana pipiens*) possessing strong cytostatic and cytotoxic activity. The active component of this material turned to be a small basic protein (molecular weight 11820, isoelectric point 9.7) that was also present in unfertilized oocytes. Amino acid sequencing of this protein, initially named P-30 Protein, later Onconase or ranpirnase, demonstrated the sequence similarity to enzymes within the superfamily of RNase A (Ardelt et al., 1991). Indeed, the protein was able to degrade RNA and other RNase substrates (Ardelt et al., 1991; Ardelt et al., 1994) and was highly cytotoxic to cancers cells of several cell lines (Darzynkiewicz et al., 1988). As it was later reported, on molar basis Onconase was 10–30 fold more cytotoxic than BS-RNase (Matousek et al. 2003; Mosimann, et al. 1994). With its single chain of 104 amino acid residues (20 residues less than RNase A) Onconase remains the smallest enzyme in the RNase A superfamily. It is also the first RNase tested in clinical trials in the U.S. and Europe, recently in phase III for unresectable malignant mesothelioma.

Onconase was previously a subject of review articles discussing its structure and function, mechanisms of cytotoxicity and therapeutic potentials (Ardelt et al., 2008; Lee and Raines, 2008; Saxena et al., 2003). It was also reviewed together with other cytotoxic RNases or other anticancer agents (Arnold and Ulbrich-Hofmann, 2006; Benito et al., 2005; Leland and Raines, 2001; Lu et al. 2008; Makarov and Ilinskaya, 2003; Makarov et al., 2008; Matoušek, 2001; Ramos-Nino, 2007; Rybak and Newton, 1999; Youle and D'Alessio, 1997).

More recently, another cytotoxic RNase was discovered by Alfacell Corporation in the same source (Ardelt et al., 2002; Singh et al., 2007) and named Amphinase. This enzyme is another homologue of pancreatic type RNases, more basic than Onconase and the largest among frog RNases, with 10 amino acid residues more than Onconase.

3.1 Structures and functions

3.1.1 Amino acid sequences and three-dimensional structures—Onconase isolated from oocytes is polymorphic at position 25. Thr is present in approximately 70 % of its molecules and Ser in the remaining 30 % (Ardelt et al., 2008). Both forms are equivalent in respect to catalytic activity and cytotoxicity. A more basic variant of Onconase with three amino acid replacements (Ile11Val, Asp20Asn, Ser103Arg-Onconase) was also isolated from oocytes and characterized (Ardelt et al., 1996). The presence of genes encoding Thr25-Onconase and another variant: Ile11Leu, Asp20Asn, Lys85Thr-Onconase was also reported by Liao et al. (2003) and Chen et al. (2000), respectively. Thus, *Rana pipiens* genome seems

to contain at least four genes coding for Onconase variants with amino acid replacements at positions: 11, 20, 25, 85 and 103.

The genome also contains at least four genes encoding Amphinase. Four variants of this enzyme denoted Amphinase 1–4 (according to the order of their elution from a reversed phase HPLC column) were found in the frog eggs (Ardelt et al., 2000; Singh et al. 2007). Their amino acid sequences demonstrate 95 (of 114) invariant residues (83 % conservation). Variants 1 and 2 differ by one residue only. Other variants differ from one another by 12–15 residues (86.8–89.9% identity) at 19 polymorphic positions. Amphinase variants are 38.2–40.0% identical with Onconase, 40.7–42.5% with *Rana catesbeiana* ribonuclease (RC-RNase) and 24.8–28.0% with RNase A (Singh et al., 2007). Unlike Onconase which contains a single putative site for glycosylation (Asn69) but no glyco-forms were identified, Amphinase variants are glycosylated at two sites, Asn27 (Asn25 in variant 3) and Asn91. They are the only known glycoproteins among frog RNases. Molecular weights and isoelectric points of their protein moieties are 12968 to 13077 and 9.95 to 10.16, depending on the variant. Sequence alignments of Onconase, Amphinase and other enzymes of RNase A superfamily were presented and discussed previously (Ardelt et al., 1991, 2008; Dyer and Rosenberg, 2006; Saxena et al., 2003).

Onconase and Amphinase, like most cytotoxic RNases, are small, very basic single chain (except dimeric BS-RNase) proteins. As we discuss later, cationic charge seems to be another, in addition to resistance to RNase inhibitor, prerequisite for the cytotoxicity of RNases. Catalytic residues of RNase A, His12, Lys41 and His119 (Raines, 1998) are conserved in all variants of Onconase: His10, Lys31 and His97 (Ardelt et al., 1991) and Amphinase: His15, Lys42 and His107 (Ardelt et al., 2002). According to amino acid sequencing and crystallographic studies (Mosimann et al., 1994; Singh et al., 2007) three of four disulfide bridges of RNase A (positions 26–84, 40–95 and 58–110) are conserved in Onconase at positions 19–68, 30–75 and 48–90 as well as in Amphinase (26–19, 41–85 and 56–100). The 65–72 disulfide bond of RNase A is not conserved but, Onconase and Amphinase have another, C-terminal disulfide bond, Cys 87-Cys104 or Cys97-Cys114, respectively, that is common to all frog RNases.

The N-terminal residue in Onconase is a pyroglutamic acid that is an integral part of the enzyme active site (Ardelt et al, 1991; Mosimann et al., 1994) and contributes 20-fold to k_{cat}/K_M for the reactions catalyzed by this enzyme (Lee and Raines, 2003). It is conserved in all known frog RNases except Amphinase that have a highly polar N-terminal extension segment of six amino acid residues (Singh et al., 2008). Pyroglutamic acid is a product of cyclization of Gln. This may be spontaneous, like during the recombinant production (Boix et al., 1996; Leland et al., 1998; Natomista et al., 1999) or catalyzed by glutaminyl cyclase. Most probably the cyclization takes place in the endoplasmic reticulum during the initial stages of oxidative folding and may be co-translational mechanism (Welker et al., 2007).

The general folds of Onconase (Mosimann et al., 1994) and Amphinase-2 (Singh et al., 2007) are very similar and adopt the classic bi-lobal, V or kidney shaped topology of RNase A (Raines, 1998; Richardson, 1981) formed by two 3-stranded anti-parallel β -sheets and three α -helices. Helices α_2 and α_3 flank the two β -sheets and helix α_1 is located between the β -sheets. Segments of secondary structure are similar in the three enzymes except that the β_2 strand of RNase A is not conserved in the frog RNases (that is replaced by a short loop) and Amphinase-2 has an additional, short β -strand designated β_0 , adjacent to helix α_1 (Singh et al. 2007). N-terminal pyroglutamic acid together with Asp2 folds back against helix α_1 of Onc and forms hydrogen bonds with Val96 and Lys9 in the active site (Mosimann et al., 1994). Other residues forming the active site of this enzyme are His10, Lys31 and His97 (the catalytic triad) as well as Thr35 and Phe98. The apparent role of the N-terminal pyroglutamic acid is stabilization of

Lys9 which together with Lys31 contribute 10^3 -fold to k_{cat}/K_M values but, do not seem to participate in substrate binding (Lee and Raines, 2003).

Crystallographic studies of Onconase in complex with nucleotide d(AUGA), by Lee et al. (2008) revealed that catalytic His97 assumes different conformation than analogous His residues in other RNases and also different than that in free Onconase (Mosimann et al., 1994). Imidazole ring of this residue is rotated in the complex by almost 180° in a way that its $N^{\delta 2}$ rather than $N^{\delta 1}$ provides a proton for catalysis. The $N^{\delta 1}$ is engaged in a hydrogen bond with Thr89. The classic bell shape of the k_{cat}/K_M dependence on pH indicates that both His10 and His 97 participate in catalysis by Onconase (Lee et al., 2008).

Crystallographic structures of natural and recombinant Amphinase-2 revealed that the active site of this enzyme is formed by His 15, Lys42 and His107 (the catalytic triad) as well as Lys14 and Phe108 (Singh et al., 2007). In this enzyme also, the environment of His 107 (analogous to His 97 in Onc and His 119 in RNase A) is substantially different than in most other RNase A homologues. The mobility of this residue is not restricted by hydrogen bonding to neighboring amino acids. Lys42 and Lys14 of Amph-2 are equivalent to Lys31 and Lys 9 in Onc. Lys 14 is stabilized at different position than Lys9 in Onc by a network of salt bridges and reorients the side chain of Lys42 from the active site to solvent.

RNase A homologues interact with RNA substrates not only at active sites but also through other subsites binding phosphoryl groups (P subsites) and nucleobases (B subsites) (reviewed by Pares et al., 1991; Raines, 1998). The enzymes catalyze cleavages of the P–O^{5'} bonds in nucleotides bound at the B1–P1–B2 subsites (Lee et al., 2008). B1, the pyrimidine binding subsite of Onconase is constituted by Lys33, Thr35, Asp67 and Phe68. They are proximal to uracil nucleobase (Lee et al., 2008). In Amphinase-2 Ile 44 replaces Lys33 of Onc and Thr78 is equivalent to Onc Asp67 at this subsite (Singh et al., 2007). B2, the purine binding subsites in Onconase and Amphinase-2 are arranged differently than in most members of the RNase A family because of several deletions in these regions. In the Onconase-d(AUGA) complex, Glu91 forms two hydrogen bonds with guanine and Thr89 is in close proximity to this nucleobase (Lee et al., 2008). As mentioned before, the second catalytic His residue at position 97 of this enzyme assumes an atypical conformation and does not seem to contact guanine.

Highly conserved among RNase A homologues component of the B2 site: glutamic acid residue (Glu91 in Onconase and Glu111 in RNase A) is replaced by Arg101 in Amph. However, in Amphinase-2, Arg101 is positioned away of the B2 subsite (Singh et al., 2007). Glu7, a new residue in this region, specific for this enzyme, seems to be capable of interaction with the guanine bound at this subsite. Thus, this residue could possibly take a role of Onconase Glu91. Thus, explanation of purine binding by Amphinase awaits structure refinement of this enzyme in complex with nucleic acid.

3.1.2 Unusual conformational stability of Onconase—Onconase is an exceptionally stable protein. Its midpoints of the thermal or guanidine induced transitions were around 90°C or 4.4 M, respectively (Arnold et al., 2006; Leland et al., 1998; Notomista et al., 2000; Notomista et al., 2001). Respective values for RNase A, 62.4°C (Notomista et al., 2000) and 2.8 M (Leich et al., 2006) were distinctly lower. As a consequence of high conformational stability, Onconase is also remarkably resistant to proteolysis Notomista et al. (2000).

The unusual thermodynamic stability of Onconase is to large extent due to the C-terminal disulfide bond (common to all frog RNases) and to a lesser degree to the N-terminal network of hydrogen bonds (Arnold et al., 2006; Leland et al., 2000; Notomista et al., 2001), interactions within the hydrophobic cluster (Val17, Ile22, Met23, Leu27, Phe28, and Phe36) (Arnold et al., 2006; Kolbanovskaya et al., 2000) and the absence of *cis* isomers of Pro residues (Arnold et

al., 2006; Pradeep et al., 2006). Folding studies provide further explanation of high conformational stability of this protein. Experiments with disulfide bonds intact revealed 10^3 -fold slower unfolding and 5-fold faster refolding rate as compared to those of RNase A (Arnold et al., 2006; Pradeep et al., 2006). Studies of reductive unfolding and oxidative refolding revealed more differences between the two enzymes. The 30–75 disulfide bond in Onconase is more solvent exposed than other disulfide bonds in this enzyme or in RNase A (Narayan et al., 2004). Therefore, the reduction rate of this bond is 10^3 -fold greater than that of the analogous 40–95 bond in RNase A (Xu et al., 2006). This disulfide bond substantially contributes to the enzyme stability since the des(30–75) folding intermediate (Gahl and Scheraga, 2009) and a recombinant variant, with Ala residues replacing Cys30 and Cys75 (Torrent et al., 2008) are less stable (T_m values approximately 71°C). Catalytic efficiency of this variant was reduced by 56% but, surprisingly, its cytotoxicity was only slightly affected. Overall oxidative refolding of Onconase is, however, approximately 10^2 -fold faster than that of RNase A (Gahl et al., 2004) and generates three intermediates, des(19–68, 30–75), des(30–75) and des19–68 (Gahl et al., 2008; Gahl and Scheraga, 2009; Xu et al., 2003). Thermodynamic stabilities of these intermediates and their roles in the kinetic folding pathways were recently determined by Gahl and Scheraga (2009). Thus, the complete mechanism of the Onconase oxidative folding has been deciphered. This enzyme was found substantially more efficient than RNase A at recovering its catalytically and biologically active structure. Exhaustive discussion of RNase folding mechanisms may be found in Gahl and Scheraga (2009).

3.1.3 Catalytic activity—Initial studies have shown that Onconase is 10^2 – 10^5 -fold less active against highly polymerized, single stranded RNA than RNase A (Ardelt et al., 1991, 1994; Boix et al., 1996) and Amphinase is 10^2 -fold less active than Onconase (Singh et al., 2007). Both, catalytic and antitumor activities of these enzymes can be abolished by alkylation of histidine residues (Ardelt et al., 1991; Boix et al., 1996; Singh et al., 2007; Wu et al., 1993) but, the enzymes practically do not interact with mammalian ribonuclease inhibitor (Boix et al., 1996; Kelemen et al., 1999; Leland et al., 1998; Singh et al., 2007). Thus, cytotoxicity of these enzymes like that of BS-RNase (section 2), depends on the catalysis of phosphodiester bonds cleavage inside mammalian cells. Again, similarly to BS-RNase, both enzymes evade the RNase inhibitor in cell cytosol. The apparent reason for the extremely low sensitivity of Onconase (or two other amphibian RNases discussed in section 4) to the inhibitor is lack in their sequences of many residues of RNase A important for the interaction (Leland and Raines, 2001).

Onconase is 100-fold more active towards polyuridylic than polycytidylic acids and 10-fold more active against uridylyl 3',5'-guanosine than cytidylyl 3',5'-guanosine. Thus, the enzyme has strong preference for uracil at the pyrimidine (B1) subsite and for guanine at the purine (B2) subsite. This specificity was recently confirmed (Singh et al., 2007) using highly sensitive, fluorogenic substrates: 6-FAM-dArC(dA)₂-6-TAMRA (rCA), 6-FAM-dArU(dA)₂-6-TAMRA (rUA) or 6-FAM-dArUdGdA-6-TAMRA (rUG). Onc was 59- or 850-fold more active against rUG (the substrate optimized for this enzyme by Kelemen et al., 1999) than towards rUA or rCA, respectively but it was still 82-fold slower than RNase A. On the contrary, Amphinase and RNase A have very little ability to discriminate between those substrates. However, the values of k_{cat}/K_M for Amphinase variants were approximately four orders of magnitude lower than those of RNase A (Singh et al., 2007).

The structural basis for Onconase base preference was recently explained by Lee et al. (2008). Surprisingly, the enzyme does not follow this specificity when degrading one of its natural substrates. Transfer RNA was reported as an apparent primary target of this enzyme in cells (Iordanov et al., 2000; Lin et al., 1994; Saxena et al., 2002; Suhasini and Sirdeshmukh, 2006). Interestingly, Onconase degrades tRNA with different base specificity than that determined with synthetic substrates. Suhasini and Sirdeshmukh (2006, 2007) demonstrated

preferential cleavages of some natural tRNAs between two guanine nucleobases in the UGG sequences present in the structurally important regions: the variable loop or the D-arm. Some minor cleavages occurred at GU and CU, in accordance to the originally determined base specificity of the enzyme. The same cuts were made when denatured tRNAs were digested but the GG cleavages were no longer preferable. It was, therefore, speculated that Onconase recognizes some native structural elements of tRNA for preferential cleavages. The other cleavage sites become available for the enzyme after substrate structure is damaged or lost as a result of preferential cleavages (Suhasini and Sirdeshmukh, 2006). The enzyme has not degraded GG bonds in two synthetic substrates 6-FAM-dUrGdGdA-6-TAMRA and 6-FAM-dArGdGdA-6-TAMRA designed to study this issue (Lee et al., 2008). According to these authors, binding of guanine at the B1 subsite seems improbable since this site is highly similar to those of other RNase A homologues. It seems that some yet unknown structural elements of the substrate may be involved (Lee et al., 2008; Suhasini and Sirdeshmukh, 2006, 2007). Most recently, Saxena et al. (2009) reported the degradation of double stranded RNA by Onconase. This finding is of particular importance in view of our earlier indications that the antitumor RNases may target RNA interference system (Ardelt et al., 2003; Zaho et al., 2008). We discuss this issue later in this article.

4. Other frog ribonucleases

It was early observed that several frog lectins could selectively agglutinate cancer cells (Kawauchi et al., 1975; Sakakibara et al., 1976). The amino acid sequence of sialic acid binding lectin from eggs of *Rana catesbeiana* (Titani et al., 1987) was later found similar to that of RNase A by Lewis et al., 1989 (this enzyme was later named RC-RNase). Another homologous lectin was isolated from oocytes of *Rana japonica* (Kawauchi et al., 1975) and sequenced (Titani et al., 1990). Another RNase was also found in *Rana catesbeiana* liver (Nagano et al., 1976). Its sequence (Nitta et al., 1989) was highly similar to that of RC-RNase and had very low ability to agglutinate cancer cells (Onconase discussed in section 3, did not agglutinate cells at all (Ardelt et al., 1991)). More recently Liao et al. (2000) purified and cloned five more (in addition to RC-RNase) cytotoxic RNases in *R. catesbeiana*. Their evolution was discussed by Rosenberg et al. (2001). All those RNases clearly belong to the superfamily of RNase A. For sequence alignments see Leland and Raines (2001), Singh et al. (2007), Rosenberg, et al. (2001) and Youle and D'Alessio (1997).

RNases from *R. catesbeiana* and *R. japonica* share the characteristic N-terminal pyroglutamic acid residue with Onconase and have Cys residues and the catalytic triad conserved at homologous positions. Amino acid sequences of RC-RNase and Onconase are about 50% identical. Crystallographic structure of RC-RNase. d(ACGA) complex (Leu et al., 2003) revealed more similarities. Eight Cys residues form four disulfide bonds analogous to those in Onconase. N-terminal pyroglutamic acid, Lys-9, His-10, Lys-35, and His-103 constitute the active site. In B1, pyrimidine binding subsite, Thr70 and Thr39 form hydrogen bonds with cytosine rather than with uracil that is preferred in this subsite of Onconase. Both enzymes prefer guanine at their B2 subsites that in RC-RNase is formed by Lys95, Glu97 and N-terminal pyroglutamic acid. Like Onconase, RNases from *R. catesbeiana* and *R. japonica* have high conformational stability. The T_m for RC-RNase is higher than 75 C (Leland et al., 2001). This enzyme is also not inhibited by ribonuclease inhibitor (Nitta et al., 1993). Both RNases are selectively cytotoxic to cancer cells (Liao et al., 1996; Nitta et al., 1994) and cytotoxicity of RC-RNase depends on its catalytic activity (Huang et al., 1998).

5. Binase and other microbial ribonucleases

Microbial RNases, especially binase from *Bacillus intermedius* and RNase Sa from *Streptomyces aureofaciens* were recently proposed as potential anticancer agents (Makarow

et al., 2008). Both enzymes belong to the family of RNase T1, subfamily of barnase (see Irie, 1997 for review) and are very well characterized proteins. Binase (109 amino acid residues) was sequenced by Aphanasenko et al. (1979) and its three-dimensional structure was refined by Pavlovsky et al. (1983) and Polyakow et al. (2002). RNase Sa is even smaller protein (96 residues) (Shlyapnikov et al., 1986). Its crystallographic structure, also in complexes with ligands was determined (Sevcik et al., 1991, 1993). The structure of its isoenzyme Sa3 was also refined (Sevcik et al., 2002).

Structurally, binase and RNase Sa are very different than Onconase and other members of RNase A superfamily. They are not affected by mammalian RNase inhibitor (Sevcik et al., 2002). Both enzymes are guanine-specific; the guanine binding site was well characterized by crystallography. Critical amino acid residues in the active sites are: Lys26, Glu72, Arg82, Arg86 and His101 for binase and Asn42, Glu44, Glu57 and His88 for RNase Sa, Glu and His being catalytic residues (Sevcik et al., 1990).

Binase was found cytotoxic, able to elicit apoptosis in cancer cells (Ilinskaya et al., 2007, 2008). RNase Sa was inactive against K-562 cells but its isoenzyme Sa3 demonstrated substantial cytotoxicity with IC_{50} 10-fold higher than that of Onconase (Sevcik et al., 2002). Binase is somewhat less cytotoxic than RNase Sa3 (Ilinskaya et al., 2004, 2008). RNase Sa may be made cytotoxic by cationization (Ilinskaya et al., 2002, 2004). A highly cationic variant with isoelectric point 10.2, produced by replacing negatively charged residues with Lys, exhibited cytotoxic activity of the same order of magnitude as that of Onconase to human embryonic kidney cells (Ilinskaya et al., 2004). However, binase and RNase Sa do not demonstrate significant selectivity towards cancer cells. The cationized Sa variant was cytotoxic both to normal and *v-ras* transformed mouse fibroblasts (Ilinskaya et al., 2002). Binase was more cytotoxic to fibroblasts transformed with *ras* oncogene but less cytotoxic to the same cells transformed with *src* or *fms* oncogenes as compared to normal cells (Ilinskaya et al., 2001). Therefore, the apparent lack of distinct specificity to cancer cells will be the main challenge in the development of these enzymes as drugs, as it has been for fungal ribotoxins (recently reviewed by Carreras-Sangra et al., 2008).

6. Engineered ribonuclease cytotoxicity and targeting therapies

Some RNases devoid of innate anticancer activity may be rendered cytotoxic by appropriate site-directed mutagenesis. The Raines group succeeded in designing variants of RNase A and its mammalian homologues that were no longer sensitive to inhibition by mammalian protein RNase inhibitor and, therefore, cytotoxic to cancer cells (reviewed by Rutkoski and Raines, 2008). The same effect could be also achieved by oligomerization (a natural prototype of such RNases is dimeric bovine seminal plasma RNase discussed in section 2) (see Libonati et al., 2008; Rutkoski and Raines, 2008, for review).

As mentioned earlier in section 5 of this article, cytotoxic activity of RNases may be substantially potentiated by cationization to enhance their delivery to cancer cells. This may be done by either site directed mutagenesis (Ilinskaya et al., 2002, 2004; Johnson et al., 2007) or chemical modification (reviewed by Futami and Yamada, 2008).

Antitumor activity of RNases may be also greatly enhanced by targeting them to specific cancer antigens by chemical conjugation to antibodies or by construction of appropriate fusion proteins by recombinant DNA technology (immunoRNases). Proteins other than antibodies or selected peptides were also investigated as potential targeting moieties. This research field was extensively discussed in the recent review articles (De Lorenzo and D'Alessio, 2008; Krauss et al., 2008; Rybak, 2008).

7. Cytostatic and cytotoxic properties of Onconase and Amphinase

7.1 Early studies, synergism of Onconase with other anticancer drugs

The cytostatic and cytotoxic properties of Onconase were initially observed in studies on human leukemic HL-60, submaxillary carcinoma A-253 and colon adenocarcinoma Colo 320 CM cell lines (Darzynkiewicz et al., 1988). The proliferation rate of these cells was suppressed due to a prolongation of G₁ phase of the cell cycle concomitant with a decline in frequency of DNA replicating cells. The cytotoxicity, seen as an induction of apoptosis and as reduction of cells reproductive capability in the clonogenicity tests, was concentration (10 – 100 nM), and time dependent. Interestingly, unlike most chemotherapeutic agents that act rather rapidly the cytostatic and cytotoxic effects of Onconase become apparent after a 24 – 48 h delay. The initial report on *in vivo* effect of Onconase revealed that some treated mice bearing M109 Madison carcinoma had a 12-fold longer survival than animals of the control group (Mikulski et al., 1990a).

Strong synergism was reported in early studies when Onconase was *in vitro* combined with tamoxifen or trifluoroperazine (Stelazine) (Mikulski et al., 1990b) or lovastatin (Mikulski et al., 1992) to treat pulmonary carcinoma A549 or pancreatic adenocarcinoma ASPC-1 cells. These observations prompted further studies that revealed synergism of Onconase in combination with other agents such as vincristine (Rybak et al., 1996), tumor necrosis factor α (Deptala et al., 1998), interferons (Tang et al., 2005; Tsai et al., 2002), ionizing radiation (Lee et al., 2007a), differentiation-inducing agents (Halicka et al., 2000), cepharanthine (Ita et al., 2008), and *in vivo*, with tamoxifen (Lee et al., 2003a). The cytotoxicity of Onconase was also enhanced by mild (39.0 °C) hyperthermia (Halicka et al., 2007). Of interest, and of potential importance for clinical applications of the enzyme, the observed synergisms were seen when Onconase was combined with antitumor agents each characterized by entirely *different mechanism* of action, and with some of them having very low toxicity.

7.2. Internalization and intracellular targets of Onconase

Onconase, as well as other cytotoxic ribonucleases targets intracellular RNA and thus have to be internalized. Although presence of receptors on plasma membrane was initially indicated (Wu et al., 1993), further studies found that Onconase binds to the cell surface in non-saturable way consistent with absence of specific receptors (Haigis and Raines, 2003). It was suggested therefore that the cationic Onconase binds to plasma membrane (which due to the presence of sialic acid has an anionic charge) electrostatically (Johnson et al., 2007). Since surface of most cancer types is more electronegative compared to normal cells (Marquez et al., 2004) such mechanism of the enzyme binding may contribute to its anticancer properties (Johnson et al., 2007). Consistent with this mechanism are observations of Ilinskaya et al., (2004) that the bacterial RNase binase, which is preferentially cytotoxic to tumor cells, is also strongly cationic. Internalization of Onconase occurs by energy-dependent endocytosis (Haigis and Raines, 2003) that is mediated by AP-2/clathrin, i.e. the mechanism dependent on clathrin adaptors and the GTPase dynamin (Rodriguez et al., 2007). From endocytes Onconase accesses cytosol likely bypassing the Golgi or endoplasmic reticulum organelles (Haigis and Raines, 2003).

Since Onconase or Amphinase chemically modified to extinguish their enzymatic activity, although able to enter the cell, are ineffective (Wu et al., 1993; Ardelt et al., 1991, 2008) the intracellular RNA has to be their target whose destruction leads to the observed cytostatic/ cytotoxic effects. Upon entrance to the cell the ribonucleolytic activity of Onconase is sustained by two already discussed in this article factors. One is its exceptional resistance to RNase inhibitor, the other is conformational stability. The stability prevents Onconase conformational

changes that otherwise could incur upon interactions with cytosolic constituents within the environment of diverse intracellular compartments.

It was initially postulated that cellular 28 S and 18 S rRNA is the primary Onconase targets (Wu et al., 1993). Degradation of tRNA was subsequently proposed as the mechanism of the enzyme activity (Saxena et al., 2002). As mentioned earlier, the Onconase-cleavage sites on tRNA have been identified as the bonds between GG and UG bases (Suhasini and Sirdeshmukh, 2006). Degradation of tRNA or rRNA leads to rather indiscriminate inhibition of translation. Several observations, however, pertinent to specific effects of Onconase, are incompatible with this mechanism. First, the overall pattern and kinetics of changes in the treated cells are different than the changes seen in cells treated with translation inhibitors such as cycloheximide (Gong, et al., 1993). While the latter kill cells indiscriminately in the cell cycle, Onconase initially induces G₁ arrest and apoptosis occurs with a 24 – 48 h delay (Juan et al., 1998). Furthermore, the proteins coded by several genes, some of them involved in regulation of cell cycle progression, are in fact up-regulated after treatment with Onconase (Juan et al., 1998). The latter finding is incompatible with the mechanism in which tRNA, mRNA and/or rRNA, either individually or collectively, are the critical targets. If this would be the case, an overall non-selective suppression of protein synthesis would take place rather than the up-regulation of individual proteins, as observed. We postulated, therefore, that one of the targets of Onconase is the non-coding RNA (microRNAs) that is involved in regulation of gene expression through RNA interference (RNAi) (Ardelt et al., 2003).

7.3. Possible mechanisms of anticancer activity of Onconase and Amphinase

The non-coding RNA (microRNAs) indeed can be the target of Onconase. Specifically, we have recently observed that the silencing of the glyceraldehyde 3-phosphate dehydrogenase gene in human lung adenocarcinoma A549 cells by siRNA was effectively prevented by Onconase (Zhao et al., 2008). The data provided evidence that siRNA, likely within the RNA-induced silencing complex, was destroyed by the enzyme, at least to the point that translation of the message was restored. Since development of many tumors is associated with early alterations at the level of microRNA genes and these genes are located in the genome hot spots associated with cancer (Calin and Croce, 2006; Volinia et al., 2006), targeting RNAi may be one of the mechanisms responsible for anticancer activity of Onconase. The observed synergisms of the enzyme with several antitumor agents may also be a consequence of Onconase targeting RNAi, specifically the family of microRNAs shown to enhance tumor resistance to cytotoxic anticancer therapy *via* mobilizing the cell defense mechanisms (Kovalchuk et al., 2008; Weidhaas et al., 2007; Yang et al., 2008).

Targeting RNAi does not exclude a possibility that other RNAs in cancer cells may also be sensitive to Onconase and that their destruction may have the observed anticancer effects. An attractive target may be RNA associated with telomerase. Telomeres are replenished by RNA-templated synthesis of telomeric DNA. Strong evidence indicates that shortening telomerase RNA induces senescence of tumor cells thereby limiting their “immortality” (Feldser and Greiger, 2007). Arrest of cancer cells in G₁ concomitant with upregulation of p21^{WAF1} after treatment with Onconase (Juan et al., 1998) is consistent with the induction of cell senescence. In fact the induction of p21^{WAF1} is one of the hallmarks and prerequisite of cell senescence (Perucca et al., 2009).

The dsRNA-dependent protein kinase R, the enzyme that phosphorylates IκB and this way activates the ubiquitous transcription factor NFκB (Bassers and Baldwin, 2006; McKenna et al., 2007) is another potential target of Onconase that may confer the properties to enhance tumor sensitivity to other anticancer drugs. Among numerous genes activated by NFκB are the “survival genes” that regulate cell growth and protect cells from apoptosis providing drug-resistance (Bessers and Baldwin, 2006). One mechanism by which the enzyme could prevent

the induction of NF- κ B is direct targeting the mRNA coding for this transcription factor. Another mechanism may involve degradation of dsRNA which is a cofactor of the dsRNA-dependent protein kinase R. Consistent with this mechanism are our findings of suppression of NF κ B activity concomitant with arrest in the cell cycle of Jurkat cells treated with Onconase (Tsai et al., 2002) and enhancement of the cytotoxicity of TNF α (Deptala et al., 1998).

Prolonged cells exposure to Onconase or to Amphinase leads to apoptosis (Ardelt et al., 2007; Darzynkiewicz et al., 1988; Deptala et al., 1998). It is manifested by typical morphologic changes such as cell shrinkage, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies, changes considered to be the hallmarks of apoptotic mode of cell death (Darzynkiewicz et al., 1997). In some instances, however, Onconase was reported to induce caspase-independent cell death, with features resembling autophagy (Michaelis et al., 2007), or cell senescence (Juan et al., 1998).

As outlined above Onconase is internalized and may have several intracellular targets including tRNA, rRNA, mRNA, and as recently shown, the non-coding RNA (microRNAs). It is possible therefore that depending on the cell (tumor) type the enzyme, when used as a single agent, may preferentially target different RNA species. The sensitivity of different tumors to Onconase thus may vary depending: (i) on the particular RNA that is preferentially degraded in the tumor, and (ii) on how essential for cell survival is the targeted RNA. In fact, significant variation in sensitivity was observed when cytotoxicity of Onconase was tested on the National Cancer Institute's Developmental Therapeutics Program (NCI-60) sixty cancer cell lines of diverse lineage (unpublished).

It should be stressed that regardless of the cell (tumor) type used in the studies Onconase augmented its sensitivity to other cytotoxic agents. The major clinical asset of Onconase thus, may be in using it as an adjunct to other therapeutic modalities in order to enhance their cytotoxic effects. Urgent studies are needed, however, to observe whether Onconase does (or does not) enhance sensitivity of normal cells as well. These studies will reveal to what extent it can improve the "therapeutic window" in application of these drugs.

8. Cytotoxic ribonucleases, Onconase, Quo Vadis?

Cytotoxic ribonucleases as exemplified by *Rana pipiens* Onconase represent a new class of antitumor agents, with an entirely different mechanism of action than the drugs currently used in the clinic. As a single agent Onconase is cytotoxic and cytostatic to a variety of tumor cell lines. Of particular importance, however, are the findings that Onconase enhances sensitivity of tumor cells to different antitumor modalities. There is a growing body of evidence that intracellular targets of Onconase are microRNAs and NF- κ B, the factors known to provide resistance of tumor cells to treatment. In clinical trials for unresectable malignant mesothelioma Onconase was shown to display antitumor activity (Beck et al., 2008; Mikulski et al., 2002; Vogelzang et al., 2005), particularly in the cases that failed a prior chemotherapy regimen. Such patients when treated with Onconase plus doxorubicin experienced a significant ($p=0.016$) prolongation of the median survival time compared to the patients treated with doxorubicin alone (Phase III Clinical Studies; data unpublished). The use of Onconase as an adjunct drug to increase the effectiveness of treatment of various tumors with chemotherapy or radiotherapy appears to be the most promising direction in its clinical applications. Additional studies on animal models including human tumors grafted on SCID mice and further clinical trials are needed to reveal clinical potential of Onconase. Further studies on animal tumor models are also needed to explore anticancer properties of other ribonucleases such as *Rana pipiens* Amphinase (Ardelt et al., 2007; Singh et al., 2007), *Rana catesbeiana* or *Rana japonica* RNases (Youle and D'Alessio, 1997), seminal ribonuclease (D'Alessio et al., 1997;

Youle and D'Alessio, 1997), modified forms of RNase A (Rutkoski and Raines, 2008) or bacterial RNases Makarow et al., 2008).

Acknowledgments

This work was supported in part by the grant R0128705 from National Cancer Institute.

References

- Aleksandrowicz J. Intracutaneous ribonuclease in chronic myelocytic leukemia. *Lancet* 1958;2:420.
- Aleksandrowicz J, Urbanczyk J, Ostrowska A, Sierko J. Further research on the activity of ribonucleases in the blood and urine of patients suffering from proliferative hemocytopenia. *Blood* 1958;13:652–64. [PubMed: 13560564]
- Aphanasenko GA, Dudkin SM, Kaminir LB, Leshchinskaya IB, Severin ES. Primary structure of ribonuclease from *Bacillus intermedius* 7P. *FEBS Lett* 1979;97:77–80. [PubMed: 761620]
- Ardelt B, Ardelt W, Darzynkiewicz Z. Cytotoxic ribonucleases and RNA interference. *Cell Cycle* 2003;2:22–24. [PubMed: 12695680]
- Ardelt B, Ardelt W, Pozarowski P, Kunicki J, Shogen K, Darzynkiewicz Z. Cytostatic and cytotoxic properties of Amphinase, a novel cytotoxic ribonuclease from *Rana pipiens* oocytes. *Cell Cycle* 2007;6:3097–3102. [PubMed: 18073526]
- Ardelt W, Lee H-S, Randolph G, Viera A, Mikulski SM, Shogen K. Enzymatic characterization of onconase, a novel ribonuclease with antitumor activity. *Protein Sci* 1994;3 (Suppl 1):137.
- Ardelt, W.; Lee, H-S.; Randolph, G.; Viera, A.; Mikulski, SM.; Shogen, K. Characterization of a natural variant of onconase. The 4th International Meeting on Ribonucleases: Chemistry, Biology and Biotechnology; Groningen, The Netherlands. 1996.
- Ardelt W, Mikulski SM, Shogen K. Amino acid sequence of an anti-tumor protein from *Rana pipiens* oocytes and early embryos. Homology to pancreatic ribonucleases. *J Biol Chem* 1991;26:245–251. [PubMed: 1985896]
- Ardelt W, Shogen K, Darzynkiewicz Z. Onconase and Amphinase, the antitumor ribonucleases from *Rana pipiens* oocytes. *Curr Pharm Biotech* 2008;9:215–225.
- Arnold U. Aspects of the Cytotoxic Action of Ribonucleases. *Curr Pharmacol Biotech* 2008;9:161–168.
- Arnold U, Schulenburg C, Schmidt D, Ulbrich-Hofmann R. Contribution of structural peculiarities of Onconase to its high stability and folding kinetics. *Biochemistry* 2006;45:3580–3587. [PubMed: 16533040]
- Arnold U, Ulbrich-Hofmann R. Natural and engineered ribonucleases as potential cancer therapies. *Biotechnol Lett* 2006;28:1615–22. [PubMed: 16902846]
- Basseres DS, Baldwin AS. Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. *Oncogene* 2006;30, 25:6817–6830. [PubMed: 17072330]
- Beck AK, Pass HI, Carbone M, Yang H. Ranpirnase as a potential antitumor ribonuclease treatment for mesothelioma and other malignancies. *Future Oncol* 2008;4:341–349. [PubMed: 18518759]
- Beintema JJ, Schuller C, Irie M, Carsana A. Molecular evolution of the ribonuclease superfamily. *Prog Biophys Mol Biol* 1988;151:165–192. [PubMed: 3074337]
- Benito A, Ribó M, Vilanova M. On the track of antitumor ribonucleases. *Mol Biosyst* 2005;1:294–302. [PubMed: 16880994]
- Boix E, Wu YN, Vasandani VM, Saxena SK, Ardelt W, Ladner J, Youle RJ. Role of the N-terminus in RNase A homologues: differences in catalytic activity, ribonuclease inhibitor interaction and cytotoxicity. *J Mol Biol* 1996;257:992–1007. [PubMed: 8632481]
- Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. *Oncogene* 2006;25:6202–6210. [PubMed: 17028600]
- Capasso S, Mattia CA, Mazzarella L, Zagari A. Refinement of the structure of bovine seminal ribonuclease. *Biopolymers* 1983;22:327–332. [PubMed: 6673761]

- Carreras-Sangra N, Alvarez-Garcia E, Herrero-Galan E, Tome J, Lacadena J, Alegre-Cebollada J, Onaderra M, Gavilanes JG, Martinez-del-Pozo A. The therapeutic potential of fungal ribotoxins. *Curr Pharm Biotech* 2008;9:153–160.
- Chen SI, Leu SY, Newton DL, Maizel JV, Rybak SM. A gender-specific mRNA encoding a cytotoxic ribonuclease contains a 3' UTR of unusual length and structure. *Nucleic Acids Res* 2000;28:2375–2382. [PubMed: 10871370]
- Di Donato A, D'Alessio G. Interchain disulfide bridges in ribonuclease BS-1. *Biochem Biophys Res Commun* 1973;55:919–928. [PubMed: 4761089]
- D'Alessio G, Di Donato A, Parente A, Piccoli R. Seminal RNase: A unique member of the ribonuclease family. *Trends Biochem Sci* 1991;16:104–106. [PubMed: 2057997]
- D'Alessio, G.; Di Donato, A.; Mazzarella, L.; Piccoli, R. Seminal ribonuclease: the importance of diversity. In: D'Alessio, G.; Riordan, JF., editors. *Ribonucleases, Structures and Functions*. Academic Press; San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: 1997. p. 383-423.
- D'Alessio G, Floridi A, De Prisco R, Pinero A, Leone E. Bull semen ribonucleases 1. Purification and physico-chemical properties of the major component. *Eur J Biochem* 1972a;26:153–161.
- D'Alessio G, Parente A, Guida C, Leone E. Dimeric structure of seminal ribonuclease. *FEBS Lett* 1972b; 27:285–288.
- Darzynkiewicz Z, Carter SP, Mikulski SM, Ardelt W, Shogen K. Cytostatic and cytotoxic effects of Pannon (P-30 Protein), a novel anticancer agent. *Cell Tissue Kinet* 1988;21:169–182. [PubMed: 3224365]
- Darzynkiewicz Z, Juan G, Li X, Gorczyca W, Murakami T, Traganos F. Cytometry in cell necrobiology. Analysis of apoptosis and accidental cell death (necrosis). *Cytometry* 1997;27:1–20. [PubMed: 9000580]
- Deptala A, Halicka HD, Ardelt B, Ardelt W, Mikulski SM, Shogen K, Darzynkiewicz Z. Potentiation of tumor necrosis factor induced apoptosis by onconase. *Int J Oncol* 1998;13:11–16. [PubMed: 9625797]
- De Lorenzo C, D'Alessio G. From immunotoxins to immunoRNases. *Curr Pharm Biotech* 2008;9:210–214.
- Dostal J, Matousek J. Purification of aspermatogenic substance (AS) from bull seminal vesicle fluid. *J Reprod Fertil* 1972;31:273–275. [PubMed: 4637638]
- Dyer KD, Rosenberg HF. The RNase a superfamily: generation of diversity and innate host defense. *Mol Divers* 2006;10:585–597. [PubMed: 16969722]
- Feldser DM, Greider CW. Short telomeres limit tumor progression in vivo by inducing senescence. *Cancer Cell* 2007;11:461–469. [PubMed: 17433785]
- Gahl RF, Narayan M, Xu G, Scheraga HA. Trimethylamine-N-oxide modulates the reductive unfolding of Onconase. *Biochem Biophys Res Commun* 2004;325:707–710. [PubMed: 15541346]
- Gahl RF, Narayan M, Xu G, Scheraga HA. Dissimilarity in the oxidative folding of Onconase and ribonuclease A, two structural homologues. *Prot Eng Des Sel* 2008;21:223–231.
- Gahl RF, Scheraga HA. Oxidative folding pathway of Onconase, a ribonuclease homologue: insight into oxidative folding mechanism from a study of two homologues. *Biochemistry* 2009;48:2740–2751. [PubMed: 19309163]
- Gong J, Li X, Darzynkiewicz Z. Different patterns of apoptosis of HL-60 cells induced by cycloheximide and camptothecin. *J Cell Physiol* 1993;157:263–270. [PubMed: 8227159]
- Haigis MC, Raines RT. Secretory ribonucleases are internalized by a dynamin- independent endocytic pathway. *J Cell Sci* 2003;116 (Pt 2):313–324. [PubMed: 12482917]
- Halicka HD, Ardelt B, Shogen K, Darzynkiewicz Z. Mild hyperthermia predisposes tumor cells to undergo apoptosis upon treatment with Onconase. *Int J Oncol* 2007;30:841–847. [PubMed: 17332922]
- Halicka HD, Murakami T, Papageorgio CN, Mittelman A, Mikulski SM, Shogen K, Darzynkiewicz Z. Induction of differentiation of leukaemic (HL-60) or prostate cancer (LNCaP, JCA-1) cells potentiates apoptosis triggered by Onconase. *Cell Proliferat* 2000;33:407–417.
- Hosokawa S, Irie M. Purification and properties of seminal vehicle ribonucleases. *J Biochem (Tokyo)* 1971;69:683–697. [PubMed: 4995443]

- Huang H-C, Wang S-C, Leu Y-J, Lu S-C, Liao Y-D. The *Rana catesbeiana* rcr gene encoding a cytotoxic ribonuclease. *J Biol Chem* 1998;273:6395–6401. [PubMed: 9497370]
- Ilinskaya ON, Decker K, Koschinski A, Dreyer F, Repp H. *Bacillus intermedius* ribonuclease as inhibitor of cell proliferation and membrane current. *Toxicology* 2002;156:101–107. [PubMed: 11164612]
- Ilinskaya ON, Dreyer F, Mitkevich VA, Shaw KL, Pace CN, Makarov AA. Changing the net charge from negative to positive makes ribonuclease Sa cytotoxic. *Protein Sci* 2002;11:2522–2525. [PubMed: 12237473]
- Ilinskaya ON, Koschinski A, Mitkevich VA, Repp H, Dreyer F, Pace CN, Makarov AA. Cytotoxicity of RNases is increased by cationization and counteracted by K_{Ca} channels. *Biochem Biophys Res Commun* 2004;314:550–554. [PubMed: 14733942]
- Ilinskaya ON, Koschinski A, Repp H, Mitkevich VA, Dreyer F, Scholtz JM, Pace CN, Makarov AA. RNase-induced apoptosis: fate of calcium-activated potassium channels. *Biochimie* 2008;90:717–725. [PubMed: 18291113]
- Johnson RJ, Chao TY, Lavis LD, Raines RT. Cytotoxic ribonucleases: the dichotomy of Coulombic forces. *Biochemistry* 2007;46:10308–10308. [PubMed: 17705507]
- Jordanov MS, Ryabinina OP, Wong J, Dinh TH, Newton DL, Rybak SM, Magun BE. Molecular determinants of apoptosis induced by the cytotoxic ribonuclease Onconase: evidence for cytotoxic mechanism different from inhibition of protein synthesis. *Cancer Res* 2000;60:1983–1994. [PubMed: 10766189]
- Ita M, Halicka HD, Tanaka T, Kurose A, Ardelt B, Shogen K, Darzynkiewicz Z. Remarkable enhancement of cytotoxicity of onconase and cepharanthine when used in combination on various tumor cell lines. *Cancer Biol Ther* 2008;7:1104–1108. [PubMed: 18443430]
- Johnson RJ, Chao TY, Lavis LD, Raines RT. Cytotoxic ribonucleases: the dichotomy of Coulombic forces. *Biochemistry* 2007;46:10308–10316. [PubMed: 17705507]
- Juan G, Ardelt B, Mikulski SM, Shogen KM, Ardelt W, Mittelman A, Darzynkiewicz Z. G₁ arrest of U-937 cells by onconase is associated with suppression of cyclin D3 expression, induction of p16^{INK4A}, p21^{WAF1/CIP1} and p27^{KIP} and decreased pRb phosphorylation. *Leukemia* 1998;12:1241–1248. [PubMed: 9697879]
- Kamaya Y, Oyama F, Oyama R, Sakakibara F, Nitta K, Kawauchi H, Takayanagi Y, Titani K. Amino acid sequence of a lectin from Japanese frog (*Rana japonica*) eggs. *J Biochem (Tokyo)* 1990;108:139–143. [PubMed: 2229005]
- Kawauchi H, Sakakibara F, Watanabe K. Agglutinins from frog eggs: a new class of proteins causing preferential agglutination of tumor cells. *Experientia* 1975;31:364–365. [PubMed: 163754]
- Kelemen BR, Klink TA, Behlke MA, Eubanks SR, Leland PA, Raines RT. Hypersensitive substrates for ribonucleases. *Nucleic Acids Res* 1999;27:3696–3701. [PubMed: 10471739]
- Kim JS, Soucek J, Matousek J, Raines RT. Catalytic activity of bovine seminal ribonuclease is essential for its immunosuppressive and other biological activities. *Biochem J* 1995a;308:547–550. [PubMed: 7772040]
- Kim JS, Soucek J, Matousek J, Raines RT. Structural basis for the biological activities of bovine seminal ribonuclease. *J Biol Chem* 1995b;270:10525–10530. [PubMed: 7737987]
- Kolbanovskaya EY, Trewisscha van Scheltinga AC, Mukhortov VG, Ardelt W, Beintema JJ, Karpeysky MY. Localization and analysis of nonpolar regions in Onconase. *Cell Mol Life Sci* 2000;57:1306–1316. [PubMed: 11028920]
- Kovalchuk O, Filkowski J, Meservy J, Ilnytsky Y, Tryndyak VP, Chekhun VF, Pogribny IP. Involvement of microRNAs-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. *Mol Cancer Ther* 2008;7:2152–2159. [PubMed: 18645025]
- Krauss J, Arndt MAE, Dubel S, Rybak SM. Antibody-targeted RNase fusion proteins (immunoRNases) for cancer therapy. *Curr Pharm Biotech* 2008;9:231–234.
- Ledoux L. Action of ribonuclease on certain ascites tumours. *Nature* 1955a;174:258–259.
- Ledoux L. Action of ribonuclease on two solid tumours in vivo. *Nature* 1955b;176:36–37. [PubMed: 14394135]
- Ledoux L. Action of ribonuclease on neoplastic growth. II Action on Landschutz ascites cells in vitro. *Biochim Biophys Acta* 1956;20:369–377. [PubMed: 13328862]

- Ledoux L, Baltus E. Action de la ribonuclease sur les cellules du carcinoma d'Erlich. *Experientia* 1954;10:500–501. [PubMed: 14353090]
- Ledoux L, Revell SH. Action of ribonuclease on neoplastic growth. I Chemical aspects of normal tumour growth: the Landschutz ascites tumour. *Biochim Biophys Acta* 1955;18:416–426. [PubMed: 13276412]
- Lee JE, Bae E, Bingman CA, Philips GN Jr, Raines RT. Structural basis for catalysis by Onconase. *J Mol Biol* 2008;375:165–177. [PubMed: 18001769]
- Lee I, Kim DH, Sunar U, Magnitsky S, Shogen K. The therapeutic mechanisms of ranpirnase-induced enhancement of radiation response on A549 human lung cancer. *In Vivo* 2007a;21:721–728. [PubMed: 18019404]
- Lee I, Lee YH, Mikulski SM, Shogen K. Effect of ONCONASE +/- tamoxifen on ASPC-1 human pancreatic tumors in nude mice. *Adv Exp Med Biol* 2003;530:187–196. [PubMed: 14562716]
- Lee JE, Raines RT. Contribution of active-site residues to the function of onconase, a ribonuclease with antitumoral activity. *Biochemistry* 2003;42:11443–11450. [PubMed: 14516195]
- Lee JE, Raines RT. Ribonucleases as novel chemotherapeutics. The ranpirnase example. *Biodrugs* 2008;22:53–58. [PubMed: 18215091]
- Leland PA, Raines RT. Cancer chemotherapy: ribonucleases to the rescue. *Chem Biol* 2001;8:405–413. [PubMed: 11358688]
- Leland PA, Schultz LW, Kim BM, Raines RT. Ribonuclease A variants with potent cytotoxic activity. *Proc Natl Acad Sci USA* 1998;95:10407–10412. [PubMed: 9724716]
- Leland PA, Staniszewski KE, Kim B, Raines RT. A synapomorphic disulfide bond is critical for the conformational stability and cytotoxicity of an amphibian ribonuclease. *FEBS Lett* 2000;477:203–207. [PubMed: 10908721]
- Leich F, Koditz J, Ulbrich-Hofman R, Arnold U. Tandemization endows bovine pancreatic ribonuclease with cytotoxic activity. *J Mol Biol* 2006;358:1305–1313. [PubMed: 16580680]
- Leu YJ, Chern SS, Wang SC, Hsiao YY, Amiraslanov I, Liaw YC, Liao YD. Residues involved in the catalysis, base specificity, and cytotoxicity of ribonuclease from *Rana catesbeiana* based upon mutagenesis and X-ray crystallography. *J Biol Chem* 2003;278:7300–7309. [PubMed: 12499382]
- Levis M, Hunt L, Barker W. Striking sequence similarity among sialic acid-binding lectin, pancreatic ribonucleases and angiogenin: Possible structural and functional relationships. *Prot Seq Data Anal* 1989;2:101105.
- Liao YD, Huang HC, Chan HJ, Kuo SJ. Large-scale preparation of a ribonuclease from *Rana catesbeiana* (bullfrog) oocytes and characterization of its specific cytotoxic activity against tumor cells. *Prot Express Purif* 1996;7:194–202.
- Liao YD, Huang HC, Leu YJ, Wei CW, Tang PC, Wang SC. Purification and cloning of cytotoxic ribonucleases from *Rana catesbeiana* (bullfrog). *Nucleic Acids Res* 2000;28:4097–4104. [PubMed: 11058105]
- Liao YD, Wang SC, Leu YJ, Wang CF, Chang ST, Hong YT, Pan YR, Chen C. The structural integrity exerted by N-terminal pyroglutamate is crucial for the cytotoxicity of frog ribonuclease from *Rana pipiens*. *Nucleic Acids Res* 2003;31:5247–5255. [PubMed: 12954760]
- Libonati M, Gotte G, Vottariello F. A novel biological actions acquired by ribonuclease through oligomerization. *Curr Pharm Biotech* 2008;9:200–2009.
- Lin JJ, Newton DL, Mikulski SM, Kung HF, Youle RJ, Rybak SM. Characterization of the mechanism of cellular and cell free protein synthesis inhibition by an anti-tumor ribonuclease. *Biochem Biophys Res Commun* 1994;204:156–162. [PubMed: 7945354]
- Lu CX, Nan K-J, Lei Y. Agents from amphibians with anticancer properties. *Anti-cancer Drugs* 2008;19:931–939. [PubMed: 18827558]
- Makarov AA, Ilinskaya ON. Cytotoxic ribonucleases: molecular weapons and their targets. *FEBS Lett* 2003;540:15–20. [PubMed: 12681476]
- Makarov AA, Kolchinski A, Ilinskaya ON. Binase and other microbial RNases as potential anticancer agents. *BioEssays* 2008;30:789–790.
- Márquez M, Nilsson S, Lennartsson L, Liu Z, Tammela T, Raitanen M, Holmberg AR. Charge-dependent targeting: results in six tumor cell lines. *Anticancer Res* 2004;24:1347–1351. [PubMed: 15274294]

- Matoušek J. Ribonucleases and their antitumor activity. *Comp Biochem Physiol C* 2001;129:175–191.
- Matousek J, Soucek J, Slavik T, Tomanek M, Lee JE, Raines RT. Comprehensive comparison of the cytotoxic activities of onconase and bovine seminal ribonuclease. *Comp Biochem Physiol Part C* 2003;136:343–356.
- Mazzarella L, Capasso S, Demassi D, Di Lorenzo G, Mattia CA, Zagari A. Bovine seminal ribonuclease: structure at 1.9 Å resolution. *Acta Crystallogr D* 1993;49:389–402. [PubMed: 15299514]
- McKenna SA, Lindhout DA, Kim I, Liu CW, Gelev VM, Wagner G, Puglisi JD. Molecular framework for the activation of RNA-dependent protein kinase. *J Biol Chem* 2007;282:11474–11486. [PubMed: 17284445]
- Michaelis M, Cinatl J, Anad P, Rothweiler F, Kotchetkov R, von Deimling A, Doerr HW, Shogen K, Cinatl J Jr. Onconase induces caspase-independent cell death in chemoresistant neuroblastoma cells. *Cancer Lett* 2007;250:107–16. [PubMed: 17084521]
- Mikulski SM, Ardel W, Shogen K, Bernstein EH, Menduke H. Striking increase of survival of mice bearing M109 Madison carcinoma treated with a novel protein from amphibian embryos. *J Natl Cancer Inst* 1990a;82:151–153. [PubMed: 2294226]
- Mikulski SM, Constanzi JJ, Vogelzang NJ, McCachren S, Taub RN, Chun H, Mittelman A, Panella T, Puccio C, Fine R, Shogen K. Phase II trial of a single weekly intravenous dose of ranpirnase in patients with unresectable malignant mesothelioma. *J Clin Oncol* 2002;20:274–81. [PubMed: 11773179]
- Mikulski SM, Viera A, Ardel W, Menduke H, Shogen K. Tamoxifen and trifluoroperazine (Stelazine) potentiate cytostatic/cytotoxic effects of P-30 protein, a novel protein possessing anti-tumor activity. *Cell Tissue Kinet* 1990b;23:237–346. [PubMed: 2357721]
- Mikulski SM, Viera A, Darzynkiewicz Z, Shogen K. Synergism between a novel amphibian oocyte ribonuclease and lovastatin in inducing cytostatic and cytotoxic effects in human lung and pancreatic carcinoma cell lines. *Br J Cancer* 1992;66:304–310. [PubMed: 1503903]
- Mosimann SC, Ardel W, James MNG. Refined 1.7 Å x-ray crystallographic structure of P-30 Protein, an amphibian ribonuclease with anti-tumor activity. *J Mol Biol* 1994;236:1141–1153. [PubMed: 8120892]
- Narayan M, Xu G, Ripoli DR, Zhai H, Breuker K, Wanjalla C, Leung HJ, Navon A, Welker E, McLafferty FW, Scheraga HA. Dissimilarity in the reductive unfolding pathways of two ribonuclease homologues. *J Mol Biol* 2004;338:795–809. [PubMed: 15099746]
- Nitta R, Katayama N, Okabe Y, Iwama M, Watanabe H, Abe Y, Okazaki T, Oghi K, Irie M. Primary structure of a ribonuclease from Bullfrog (*Rana catesbeiana*) liver. *J Biochem* 1989;106:729–735. [PubMed: 2613682]
- Nitta K, Oyama F, Oyama R, Sekigushi K, Kawauschi H, Takayanagi Y, Hakomori SI, Titani K. Ribonuclease activity of sialic acid-binding lectin from *Rana catesbeiana* eggs. *Glycobiology* 1993;3:37–45. [PubMed: 8448385]
- Nitta K, Ozaki K, Ishikawa M, Furusawa S, Hosono M, Kawauschi H, Sasaki K, Takayanagi Y, Tsuiki S, Hakomori S. Inhibition of cell proliferation by *Rana catesbeiana* and *Rana japonica* lectins belonging to the ribonuclease superfamily. *Cancer Res* 1994;54:920–927. [PubMed: 8313382]
- Notomista E, Catanzano F, Graziano G, Dal Piaz F, Barone G, D'Alessio G, Di Donato A. Onconase: an unusually stable protein. *Biochemistry* 2000;39:8711–8718. [PubMed: 10913282]
- Notomista E, Catanzano F, Graziano G, Di Gaetano S, Barone G, Di Donato A. Contribution of chain termini to the conformational stability of onconase. *Biochemistry* 2001;40:9097–9103. [PubMed: 11478876]
- Notomista E, Cafaro V, Fusiello R, Bracale A, D'Alessio G, Di Donato A. Effective expression and purification of recombinant Onconase, an antitumor protein. *FEBS Lett* 1999;463:211–215. [PubMed: 10606723]
- Pares X, Nogues MV, de Llorens R, Cuchillo CM. Structure and function of ribonuclease A binding subsites. *Essays Biochem* 1991;26:89–103. [PubMed: 1778187]
- Pavlovsky AG, Vagin AA, Vainstein BK, Chepurnova NK, Karpeysky M Ya. Three-dimensional structure of ribonuclease from *Bacillus intermedius* 7P at 3.2 Å. *FEBS Lett* 1983;162:167–170. [PubMed: 6413257]

- Perucca P, Cazzalini O, Madide M, Savio M, Laskey RA, Vannini V, Prosperi E, Stivala LA. Loss of p21 CDKN1A impairs entry to quiescence and activates a DNA damage response in normal fibroblasts induced to quiescence. *Cell Cycle* 2009;8:105–114. [PubMed: 19106607]
- Piccoli R, Tamburini M, Picialli G, Di Donato A, Parente AD, Alessio G. The dual-mode quaternary structure of seminal RNase. *Proc Natl Acad Sci USA* 1992;89:1870–1874. [PubMed: 1542685]
- Polyakow KM, Lebedev AA, Okorokov AL, Panov KI, Schluga AA, Pavlovsky AG, Karpeisky MY, Dodson GG. The structure of substrate-free microbial ribonuclease binase and of its complexes with 3'GMP and sulfate ions. *Acta Crystallogr D* 2002;58:744–750.
- Pradeep L, Shin HC, Scheraga HA. Correlation of folding kinetics with the number and isomerization states of prolines in three homologous proteins of the RNase family. *FEBS Lett* 2006;580:5029–5032. [PubMed: 16949585]
- Raines RT. Ribonuclease A. *Chem Rev* 1998;98:1045–1065. [PubMed: 11848924]
- Ramos-Nino ME. Cytotoxic ribonuclease-based cancer therapies. *Drugs of the Future* 2007;32:1–10.
- Richardson JS. The anatomy and taxonomy of protein structure. *Adv Protein Chem* 1981;34:167–339. [PubMed: 7020376]
- Rosenberg HF, Zhang J, Liao YD, Dyer KD. Rapid diversification of RNase A superfamily ribonucleases from the bullfrog, *Rana catesbeiana*. *J Mol Evol* 2001;53:31–38. [PubMed: 11683320]
- Rodriguez M, Torrent G, Bosch M, Dubremetz JF, Ribo M, Benito A, Vilanova M, Beaumelle B. Intracellular pathway of Onconase that enables its delivery to the cytosol. *J Cell Sci* 2007;120:1405–1411. [PubMed: 17374640]
- Rybak SM. Antibody-onconase conjugates: cytotoxicity and intracellular routing. *Curr Pharm Biotech* 2008;9:226–230.
- Rybak SM, Newton DL. Natural and engineered cytotoxic ribonucleases. *Exp Cell Res* 1999;253:325–335. [PubMed: 10585255]
- Rybak SM, Pearson JW, Fogler WE, Volker K, Spence SE, Newton DL, Mikulski SM, Ardel W, Riggs CW, Kung HF, Longo DL. Enhancement of vincristine cytotoxicity in drug-resistant cells by simultaneous treatment with onconase, an antitumor ribonuclease. *J Natl Cancer Inst* 1996;88:747–753. [PubMed: 8637029]
- Sakakibara F, Takayanagi G, Kawauchi H, Watanabe K, Hakomori S. An A-like lectin of *Rana catesbeiana* eggs showing unusual reactivity. *Biochim Biophys Acta* 1976;444:386–395. [PubMed: 1085640]
- Saxena A, Saxena SK, Shogen K. Effect of Onconase on double-stranded RNA *in vitro*. *Anticancer Res* 2009;29:1067–1072. [PubMed: 19414347]
- Saxena SK, Shogen K, Ardel W. Onconase^R and its therapeutic potential. *Lab Med* 2003;34:380–387.
- Saxena SK, Sirdeshmukh R, Ardel W, Mikulski SM, Shogen K, Youle RJ. Entry into cells and selective degradation of tRNAs by a cytotoxic member of the RNase A family. *J Biol Chem* 2002;277:15142–15146. [PubMed: 11839736]
- Sevcik J, Dodson EJ, Dodson GG. Determination and restrained least-squares refinement of the structures of ribonuclease Sa and its complex with 3'-guanylic acid at 1.8 Å resolution. *Acta Crystallogr B* 1991;47:240–253. [PubMed: 1654932]
- Sevcik J, Hill CP, Dauter Z, Wilson KS. Complex of ribonuclease Sa with a cyclic nucleotide and a proposed model for the reaction intermediate. *Acta Crystallogr D* 1993;49:257–271. [PubMed: 15299531]
- Sevcik J, Sanishili RG, Pavlovsky AG, Polyakov KM. Comparison of active sites of some microbial ribonucleases: structural basis for guanylic specificity. *Trends Biochem Sci* 1990;15:158–162. [PubMed: 2160143]
- Sevcik J, Urbanikova L, Leland PA, Raines TT. X-ray structure of two crystalline forms of a *Streptomyces* ribonuclease with cytotoxic activity. *J Biol Chem* 2002;277:47325–47330. [PubMed: 12228255]
- Singh UP, Ardel W, Saxena SK, Holloway DE, Vidunas E, Lee HS, Saxena A, Shogen K, Acharya KR. Enzymatic and structural characterisation of amphinase, a novel cytotoxic ribonuclease from *Rana pipiens* oocytes. *J Mol Biol* 2007;371:93–111. [PubMed: 17560606]

- Shlyapnikov SV, Both V, Kulikow VA, Dementiev AA, Sevcik J, Zelinka J. Amino acid sequence determination of guanyl-specific ribonuclease from *Streptomyces aureofaciens*. FEBS Lett 1986;209:335–339. [PubMed: 3098582]
- Suhasini AN, Sirdeshmukh R. Transfer RNA cleavages by onconase reveal unusual cleavage sites. J Biol Chem 2006;281:12201–12209. [PubMed: 16497678]
- Suhasini AN, Sirdeshmukh R. Onconase action on tRNA(Lys3), the primer for HIV-1 reverse transcription. Biophys Res Commun 2007;363:304–309.
- Suzuki H, Parente A, Farina B, Greco L, La Montagna R, Leone E. Complete amino-acid sequence of bovine seminal ribonuclease, a dimeric protein from seminal plasma. Biol Chem Hoppe-Seyler 1987;386:1305–1312. [PubMed: 3426801]
- Tang CH, Hu CC, Wei CW, Wang JJ. Synergism of *Rana catesbeiana* ribonuclease and IFN-gamma triggers distinct death machineries in different human cancer cells. FEBS Lett 2005;579:265–270. [PubMed: 15620724]
- Telford IR, Kemp JF, Taylor EF, Yeaman MW. Effect of ribonuclease on survival of ascites tumor bearing mice. Proc Soc Exp Biol Med 1959;100:829–831. [PubMed: 13645734]
- Titani K, Kuwada M, Nitta k, Sakakibara F, Takanyangi G, Hakomori SI. Amino acid sequence of sialic acid binding lectin from frog (*Rana catesbeiana*) eggs. Biochemistry 1987;26:2189–2194. [PubMed: 3304421]
- Torrent G, Benito A, Castro J, Ribo M, Vilanova M. Contribution of the C30/C75 disulfide bond to the biological properties of onconase. Biol Chem 2008;389:1127–1136. [PubMed: 18979636]
- Tsai SY, Hsieh TC, Ardelt B, Darzynkiewicz Z, Wu JM. Combined effects of onconase and IFN-beta on proliferation, macromolecular syntheses and expression of STAT-1 in JCA-1 cancer cells. Int J Oncol 2002;20:891–896. [PubMed: 11956580]
- Vestia S, Tramontano D, Augusti-Tocco G, D'Alessio G. *In vitro* studies on selective inhibition of tumors cell growth by seminal ribonuclease. Cancer Res 1980;40:3740–3744. [PubMed: 7438057]
- Vogelzang NJ, Porta C, Mutt L. New agents in the management of advanced mesothelioma. Semin Oncol 2005;32:336–50. [PubMed: 15988688]
- Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yunaiharu N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNAs expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006;103:2257–2261. [PubMed: 16461460]
- Weidhaas JB, Babar I, Nallur SM, Trang P, Roush S, Boehm M, Gillespie E, Slack FJ. MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. Cancer Res 2007;67:11111–11116. [PubMed: 18056433]
- Welker E, Hathaway L, Xu G, Narayan M, Pradeep L, Shin HC, Scheraga HA. Oxidative folding and N-terminal cyclization of onconase. Biochemistry 2007;46:5485–5493. [PubMed: 17439243]
- Wu Y, Mikulski SM, Ardelt W, Rybak SM, Youle RJ. A cytotoxic ribonuclease. Study of the mechanism of onconase cytotoxicity. J Biol Chem 1993;268:10686–10693. [PubMed: 8486718]
- Xu G, Narayan M, Kurinov I, Ripoli DR, Welker E, Khalili M, Ealick SE, Scheraga HA. A localized specific interaction alters the unfolding pathways of structural homologues. J Am Chem Soc 2006;128:1204–1213. [PubMed: 16433537]
- Xu G, Narayan M, Welker E, Scheraga HA. A novel method to determine thermal transition curves of disulfide-containing proteins and their structured folding intermediates. Biochem Biophys Res Commun 2003;311:514–517. [PubMed: 14592446]
- Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia SV, Cheng JQ. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 2008;68:425–433. [PubMed: 18199536]
- Youle RJ, D'Alessio G. Antitumor RNases. In: D'Alessio, G.; Riordan, JF., editors. Ribonucleases, Structures and Functions. Academic Press; San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: 1997. p. 491–514.
- Zhao H, Ardelt B, Ardelt W, Shogen K, Darzynkiewicz Z. The cytotoxic ribonuclease Onconase targets RNA interference (siRNA). Cell Cycle 2008;7:3258–3261. [PubMed: 18927512]