

RNase: A Novel Enzyme For Treatment Of Cancers

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Abstract

Ribonucleases (RNase) catalyses the breakdown of RNA into smaller components. The cytotoxic effects of RNase include the RNA cleavage leading to the inhibition of the protein synthesis and the induction of apoptosis. RNases uniquely influence several functions in the tumor cell simultaneously and demonstrated the ability to overcome multi-drug resistance and to enhance the cytotoxicity of a variety of anti-cancer agents. Different RNases such as onconases, bovine seminal RNase, RNase T1, α -sarcin, RNase P, actibind and RNaseT2 have recently been studied for the treatment of different type of cancers.

INTRODUCTION

Ribonucleases are abbreviated commonly as RNase. It is a nuclease that catalyses the breakdown of RNA into smaller components. They are ubiquitous, with a very short lifespan in an unprotected environment. The cytotoxic effects of RNase include the RNA cleavage leading to the inhibition of the protein synthesis and inducing apoptosis (2). The cytotoxic effect can be produced by applying it on the outside surface of the cell but it was found that cytotoxicity increases about by 1000 times when Rnase is artificially introduced into the cytosol, indicating internalisation into the cell as the rate limiting step for toxicity (19).

There are two types Rnases, endoribonucleases and exoribonucleases. Endoribonucleases are RNase A, RNase P, RNase H, RNase III, RNase T1, RNase T2, RNase U2, RNase V1, RNase I, RNase PhyM and RNase V. Exoribonucleases includes Polynucleotide Phosphorylase (PNPase), RNase PH, RNase II, RNase R, RNase D, RNase T, oligoribonuclease, exoribonuclease I and exoribonuclease II (Table 1).

Figure 1

Table 1

RNase Type	Source	Mechanisms of action	Applications
Onconase	<i>Rana pipiens</i> oocytes and early embryos	Cleavage of the t RNA and thus inhibit the transcription and protein synthesis which suppresses the IAP, lead to the activation of the caspases and apoptosis	Human pancreatic aden carcinoma, human lung carcinoma and mesothelioma cancer.
Bovine Seminal RNase	Bovine seminal fluid	Binds to and destabilizes membrane bilayers in vitro and thus reaches the cytosol and degrade cellular RNA	Treatment of thyroid cancer
RNaseT1	<i>Aspergillus oryzae</i>	RNA cleavage leading to the inhibition of the protein synthesis and inducing apoptosis	Under clinical trials
α -Sarcin	<i>Aspergillus giganteus</i>	α - sarcin acts by cleavage of the phosphodiester bond in 28S rRNA, stops the protein synthesis	Under clinical trials
RNase P	Cultured human cells	Catalyses the hydrolytic removal of 5'- leader sequence of t-RNA leading to the inhibition of the protein synthesis and inducing apoptosis	Under clinical trials
Actibind	<i>Aspergillus niger</i>	Stops new blood vessel formation (angiogenesis) in tumors thus blocking the blood supply to the tumors	Under clinical trials
RNaseT2	Cultured human cells	Action same as actibind	Under clinical trials

USE OF DIFFERENT RNASES IN CANCER ONCONASES

Onconase is a member of the RNase A super family (1). Members of RNase A super family shares about 30% identities in the amino acid sequences. This type of RNase was isolated from northern leopard frog (*Rana pipiens*) oocytes and early embryos (16). It has cytotoxic and cytostatic effects (11) led to the development of onconase as a cancer therapeutic, particularly for the external use against

the skin cancer. Due to its quaternary structure it does not bind to the ribonuclease inhibitors and thus doesn't prevent its effective inhibition of ribonucleolytic activity and could explain why onconase is cytotoxic at low concentration while RNase A is not.

Onconase may bind to cell surface carbohydrate or they may bind to the receptors originally developed for physiologically imported molecules like polypeptide hormones or probably they move inside by endocytosis. After entering into the cell they cause the cleavage of the t RNA and thus inhibit the transcription. This inhibition of the transcription would lead to the inhibition of the protein synthesis, which ultimately suppresses the IAP. IAP is responsible for the inhibition of caspases so, there own inhibition would lead to the activation of the caspases and thus the apoptosis would be activated.

Onconases were found to induce the caspase-9 – caspase-3 cascade, which is correlated with the release of cytochrome c from the mitochondria. But interestingly very little of cytochrome release was detected and this was attributed to the lack of detectable translocation of BAX from cytosol onto mitochondria in response to the Onconases. So that means the protein inhibition is not the only mechanism. Another possible mechanism can be the release of the procaspases from the mitochondria itself (8).

BOVINE SEMINAL RNASE (BS-RNASE)

Bovine seminal RNase (BS-RNase) is a unique member of the RNase A family which exist as dimer of RNase A like subunits which are linked by the disulfide like bridges, moreover it maintains the allosteric regulation by both substrate and the reaction products at nucleotide hydrolysis phase. It is highly cytotoxic and the dimer structure is very important for its activity and if it is reduced to the monomeric structure, than it will have the ribonuclease activity but cytotoxicity will decrease. Dimeric BS-RNase enters cells by adsorptive rather than receptor-mediated endocytosis and degrades cellular RNA. Dimeric form is able to evade ribonuclease inhibitor but not the monomeric form (9). It is the only dimeric ribonuclease described so far, found to exist in the two different quaternary structures consisting of two identical monomeric units, which are made up of 124 amino acid residues.

The cytotoxicity of BS RNase is related to its quaternary structure. The cytotoxicity can manifest itself as an immunosuppressive, antitumor, embryo toxic, aspermatogenic activity each of which has the potential

therapeutic value. Among the immunosuppressive activity is most likely to be physiologically significant, since this activity may be required to suppress the bovine immune response against components of the bull seminal fluid. BS RNase induced apoptosis, which was associated with activation of initiation caspase-8 and -9 followed by activation of executioner caspase-3, leading to the proteolytic cleavage of poly (ADP-ribose) polymerase. BS-RNase have been found to be the promising tool for the treatment of thyroid cancer (3).

RNASE T1

RNase T1 is an endoribonuclease that specifically degrades single stranded RNA. It cleaves the phosphodiester bond between 3'- guanylic residues and 5'OH residues of adjacent nucleotides with the formation of corresponding intermediate 2', 3'- cyclic phosphates. The reaction products are 3' GMP and oligonucleotides with a terminal 3' GMP (17). RNase T1 does not require metal ions for the activity. It is cloned from *Aspergillus oryzae*. Its main uses are in RNA structure and the mapping studies, RNA protection assays and the removal of RNA from DNA samples.

RNase T1 is non cytotoxic because of its inability to internalize into tumor cells. So, if somehow these are introduced into the cells they can be made to act as cytotoxic. In one of the experiments, RNaseT1 has been internalized into the human tumor cells via novel gene transfer reagent (HVJ) that resulted into the cell death. Internalized RNase T1 like onconases and BS-RNases also induces the apoptotic cell death. Because of its non-specificity to the tumor cells it cannot be at present developed as an anti tumor drug. But according to one of the researches conducted RNaseT1 incorporated into the HVJ envelop vector will be unique anticancer drug if HVJ envelop vector can be targeted to the tumor cells. RNase T1 induces the apoptotic cell death. Because its cytotoxicity is not specific to tumor cells, it cannot at present be developed as an anticancer drug. However it could be unique anticancer drug if HVJ envelop vector can be targeted to tumor cells. A study of this strategy is currently in progress (20).

▮-SARCIN

▮- Sarcin is a potent polypeptide toxin (cyclising ribonuclease) of 150 residues secreted by the fungus *Aspergillus giganteus* MDH18894 that belongs to the type 1 (those having only single polypeptide chain) group of the ribosome-inactivating enzyme. It is the most significant member of the family of fungal ribotoxins that display a 3-

dimensional structure.

His50, Glu96, His137 residues of β - sarcin involved in the mechanism of catalysis. The hydrolysis of 3'-5' phosphodiester bond of the substrate yielding 2'-3' cyclic mononucleotide and then conversion of the intermediate into the corresponding 3'-monophosphate derivative as the final product of the reaction. Thus, β - sarcin acts by cleavage of the phosphodiester bond of 28S rRNA, stops the protein synthesis (12). In addition to this enzymatic activity β - sarcin interacts with the lipid bi layers promoting their fusion and leakage. Any toxin that is able to produce more than 90% of inhibition of protein synthesis may induce apoptosis.

RNASE P

RNase P is unique from other RNases in that it is the ribozyme i.e. ribonucleic acid that acts as a catalyst in the same way that the protein based enzyme would. Its function is to cleave off the extra or precursor sequence of RNA on RNA molecules. In the current cancer therapy, the main problem is to distinguish between the cancer cells and the normal cells. There are certain chimeric molecules, which are specific to the cancer cells, which can act as the specific targets and thus solving the problem (4). M1 RNA is the catalytic subunit of RNase P and this subunit catalyses the hydrolytic removal of 5'- leader sequence of t-RNA.

Studies of the substrate recognition by M1 RNA have led to the development of the strategy of gene targeting by M1 RNA (4). M1 RNA can be targeted to the mRNA simply by the addition of the so-called guide sequence at the 3'terminal. So this now becomes as M1-GS, which has mRNA as its target. Cleavage of the mRNA will not allow the formation of the fusion proteins, which are specific for the cancer cells.

The utility of the M1-GS in the cancer was shown by its use against BCR-ABL oncogene model (4). This BCR-ABL oncogene was created by the translocation of the sequences from ABL gene on chromosome 9 to the BCR gene on the chromosome 22. Two oncogenes were created BCR-ABL p190 and BCR-ABL p210. Both of these differ and have identical ABL derived sequences but differ in the number of the BCR nucleotides. These chimeric molecules so formed by the chromosomal rearrangement would be specific to the cancer cells and thus serve as the excellent targets. These BCR-ABL oncogenes are responsible for the myelogenous leukemia and acute lymphoblastic leukemia (6). BCR-ABL oncogenes inhibit the apoptosis by Bcl-2 pathway as a part of their oncogenic phenotype. Inhibition of the BCR- ABL

expression would thus reverse this phenotype and the cells die by apoptosis. It should be noted that M1-GS should target only at the junction sequences of the transcribed mRNA. If not so then the mRNA of the normal cells would be cleaved and thus the resultant damage to the normal cells would occur. This gene therapy promises to be an effective strategy for the treatment of the cancer.

Using only this BCR- ABL system model has created the RNase P technology for the inhibition of the chimeric gene products and the efficiency of the agents has not been evaluated in the animal models to our knowledge and thus the efficiency of the delivery process is still a major problem to be investigated and solved. But surely this advancement provides a new therapeutic tool for the treatment of cancer and holds some promise for more selective, non-toxic therapy in the future.

ACTIBIND AND RNASE T2

Actibind, a protein that is produced by the black molds *Aspergillus niger*, a well-known microorganism used in bio and food industry. In plants, actibind binds actin, a major component of the intracellular structure in plants, interfering with the plants' pollen tubes and halting cell growth. RNase T2, was also subsequently found to bind actin in human and animal migrating cells, such as the cells that are responsible for new blood vessel formation (angiogenesis) in tumors thus blocking the blood supply to the tumors, actibind halted the ability of malignant cells to move through the blood stream to form new metastases (14). A further plus is that actibind is not toxic to normal cells, thereby significantly minimizing the risk of side effects.

The fungal actibind and the human RNaseT2 represent the basis for a new class of drugs that could be used as a front-line therapy in the fight against cancer.

CONCLUSION

Ribonucleases (RNases) are potential anti-tumor drugs due to their cytotoxicity and uniquely influence several functions in the tumor cell simultaneously. As such, RNases have demonstrated the ability to overcome multi-drug resistance and to enhance the cytotoxicity of a variety of anti-cancer agents.

RNase (Ribonucleases) Particularly members from the RNase A and RNase T1 super families have shown promising results. Among these enzymes, Onconase, an RNase from oocytes and early embryos of the northern leopard frog (*Rana pipiens*). RNases provide an attractive

alternative to conventional antitumor chemotherapeutics. With onconase, RNaseT1, β -sarcin and RNase P are RNase-based antitumor agent are under clinical trials and further developments of discovered Rnases are required by protein engineering for efficient RNase-based anti-tumor drugs.

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