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REVIEW

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Promises of eukaryotic ribonucleases for cancer treatment: a systematic review

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Abstract

Background: Following an increasing interest in exploration of anticancer chemotherapeutic agents, ribonucleases are currently under investigations for alternative anticancer chemotherapy.

Objective: The current review scrutinizes information on the potential capability of eukaryotic ribonucleases for cancer treatment.

Methods: Predefined searching terms were applied to PubMed, Scopus, eLibrary databases and other search engines such as Google Scholar and bioRvix preprints. Twenty four research articles on eukaryotic ribonuclease were included in the review. Qualitative and quantitative information of these studies were extracted, analyzed and explained in text, tables and figures.

Results: Majority of eukaryotic ribonucleases (46%, 11/24) included in the review were extracted from various species of frogs, 21% (5/24) were from bovine sources and others were from human bodies, edible mushrooms, fungal and plant species. Molecular characteristics of eukaryotic ribonucleases were illustrated in tables and figures. According to the reports, ranpirnase with a trademark of Onconase® is the sole ribonuclease granted with an orphan and fast-track drug status by FDA, USA. Most other eukaryotic ribonucleases are undergoing various preclinical stages of research for their potential anticancer effect. Hence, the mean of half – maximal inhibitory concentrations (IC_{50}) of eukaryotic ribonucleases of several research outcomes showed their selective cytotoxicity towards cancerous cells. In some reports, pre-tumor-xenografted animals treated with ribonucleases also demonstrated diminished tumor volume, lower tumor metastasis and increased survival rates. In addition, overall safety and toxicity parameters were also indicated as tolerable by the experimental hosts. However, a single study indicated degeneration of spermatogenic epithelia in wheat leave RNase treated animals.

Conclusions: Though several clinical trials on eukaryotic ribonucleases are expected, existing results from in vitro and in vivo preclinical studies showed promising alternative chemotherapy to treat cancer diseases. Hence, further human safety and efficacy studies are still necessary to explore well established applications of eukaryotic ribonucleases in clinical medicine.

Keywords: Anticancer, Metastasis, Onconase®, RNase, Secretary Ribonucleases

Introduction

The current updates of Global Cancer Statistics of GLOBOCAN estimated 19.3 million new cancer cases and about 10.0 million new cancer deaths occurred in 2020 [1]. The global cancer burden is expected to be 28.4 million cases in 2040 which is a 47% rise from 2020. Despite the efforts undertaken to reduce the risk of cancer, it

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is the most challenging disorder of the current medicine particularly in relation to the efficacy of existing chemotherapeutics.

Ribonucleases (RNases) are currently under consideration alternative to chemotherapeutic agents for cancer treatment. RNases are obtained from different origins; eukaryotic or prokaryotic organisms. Eukaryotic sources of ribonucleases in the research pipeline includes ranpirnase (Onconase®) from oocytes of northern leopard frog *Rana pipiens*, amphinase and RC-RNase from oocytes of other frog species, bovine seminal ribonuclease (BS-RNase) from bovine seminal vesicles, RNase 3 (ECP) from human blood cells, wheat leaf neutral (WLN) RNase from cereal plants and other mammalian or plant ribonucleases [2].

Most of ribonucleases extracted from eukaryotic organisms showed promising anticancer activities at preclinical studies. Cancer cell lines and tumor-induced laboratory animals were employed to characterize the antitumor efficacy and toxicity of eukaryotic ribonucleases. However, only one of them, Ranpirnase, is granted an orphan designation status by FDA on 2007 for the treatment of unresectable malignant mesothelioma (MMe) [3]. Ranpirnase, with a trade name of Onconase®, is the first ribonuclease anticancer chemotherapy introduced in to the market.

Hence, although only a single ribonuclease from *Rana pipiens* passed in to clinical phase studies, a number of eukaryotic ribonucleases from the same sources (*Rana* frogs) and other eukaryotic organisms including fungus, edible mushrooms, crops and plants are under investigation for chemotherapeutic potential of malignancies [4–8]. However, antiproliferative, anticancer, antimetastais and other major characteristics of eukaryotic ribonucleases are not well elucidated yet. Therefore, the current review was aimed at retrieving, analyzing and summarizing existing information of anticancer efficacy of eukaryotic ribonucleases. This review shows a picture of collective antitumor potential and safety of eukaryotic ribonucleases which are involved in the research pipeline of ribonucleases.

Methods

Search strategy and screening process

After a preliminary literature search of the research question based on pre-defined population, intervention, comparison and outcomes (PICOs), concepts, keywords and MeSH terms were extracted for further application of searching strategies. Three main concepts and their synonyms were identified as follows. Concept 1: cancer (synonyms: tumor and malignancy), concept 2: eukaryotic ribonuclease (synonyms: RNase, mammalian RNase, plant RNase and fungal RNase), concept 3: cancer outcomes (survival time, time for progression, tumor volume or weight reduction, antiproliferation, anticancer, antitumor, antimetastasis, apoptosis and toxicities) were formulated. All search terms of each concept of the research question were applied to search engines of PubMed, Scopus, eLibrary (Russian scientific electronic library: http://elibrary.ru) databases and other web-based sources including Google Scholar and bioRvix preprints. Boolean operators were used during concept combination search.

A total of 1044 published articles and preprint were retrieved between January 01, 2021 and February 11, 2021 (ended at 5:32:39am) from all search engines mentioned above. All these articles were exported to EndNote X9 version 19 reference management software (Clarivate Analytics™). Sixty nine duplicated articles (based on similar title, authors, journal name, volume and number/issue of articles) were removed and 955 articles were then subjected to primary selection according to a pre-stated inclusion and exclusion criteria. Title and abstract of each article were examined for encompassing the antitumor, anticancer and antineoplastic properties of eukaryotic ribonucleases and 24 original articles and pre-print of eukaryotic RNase researches were included for data extraction. Only open accessed articles in English and Russian language were included. Articles which focused on antiviral / antiparasitic / antibacterial RNases, bacterial ribonucleases, RNases for diagnostic or etiologic functions or as molecular dicer, antibody or molecule conjugated RNase, antitumor proteins such as RNase inhibitors (RI), clinical trials reported in abstract form only and articles with limited information on eukaryotic RNase were excluded.

Data extraction and management

Basic information of experimental intervention, eukaryotic RNases, including RNase name, super family, source, PICOs, molecular weight (Da), mean of half-maximal inhibitory concentration (IC_{50}), mean of total cell number at time t-interval of treatment, mean cell viability / vitality, mean of tumor volume, type of experimental model, RNase concentration or injection dose, 1-year or 2-years survival rate and time to progression of clinical trial outcomes, tissue or organ toxicities, and safety related outcomes were extracted from text, tables and graphs of included articles.

Retrieving sequences and bioinformatics analysis

Protein sequences of known anticancer eukaryotic ribonucleases were retrieved from UniProtKB consortium. UniProtKB, universal protein knowledge base, is a large resource of protein sequences and their detailed annotation. It is a joined data from Swiss-prot, TrEMBL and

PIR in which more than half a million protein sequences have been experimentally reviewed while others remain on unreview status [9]. Entry, protein and gene names, accession number, length of protein sequence, active site positions, annotation score and source of organism of cytotoxic eukaryotic RNases included in this review were searched with a query syntax of "antitumor AND RNase" and stored in a UniProtKB basket for further analysis. Homologous sequences of eukaryotic ribonucleases were created and subjected to multiple sequence alignment. The FASTA format of 14 protein sequences of these eukaryotic ribonucleases were entered to robust phylogenetic analysis for the non-specialists (www.phylogeny.fr) with TreeDyn 198.3 program of PHYLIP package [10] and their evolutionary relationship was described. PyMOL™ 2.4.1 (Incentive Product© Schrodinger, LLC) software was also employed to show the active sites amino acid residues and three dimensional (3D) structures of some ribonucleases.

Results

Description of studies included in the review

Twenty-four original published primary studies were eligible according to the predetermined inclusion criteria (as described in Methods). All studies, included in this review, were mainly focused on potential antitumor applications of eukaryotic ribonucleases. Among these included RNase studies, 46% (11/24) were RNases isolated from various species of frogs, genus *Rana* in particular, 21% (5/24) were from bovine sources, 12.5% were from human bodies and others were from edible mushrooms or fungal agents or plant species (Table 1). Majority of antitumor RNases included in this review belongs to pancreatic ribonucleases (RNase A) family. Only two clinical trial RNase studies were included while other RNase studies were limited to preclinical stages. A single study involved only apparently healthy animals in which kidney toxicity (nephrotoxicity) was solely investigated.

Molecular characteristics of eukaryotic ribonucleases

A total of twenty protein sequence entries of eukaryotic ribonucleases variants with anticancer characteristics were retrieved from UniProt knowledge base (UniProtKB) (https://www.uniprot.org/). Among included eukaryotic RNases, the longest protein sequence was observed on WLN-RNase (1392 amino acid residues) which is originated from wheat leave while other eukaryotic RNases possess a range of 104 to 380 amino acid sequences. More than 50% of protein sequences of query syntax were well annotated with annotation scores of 4 and 5 (Table 2).

All entries of protein sequences of antitumor RNases were then subjected to multiple sequence alignment using an in-built Clustal Omega program of UniProtKB. However, phylogenetic tree analysis of the protein sequences showed six outliers. Hence, after removal of these outliers, the remaining protein sequences were realigned. These protein sequences were further highlighted using annotation and amino acid properties.

Based on the Clustal Omega [UniProtKB] multiple sequence alignment of query sequences, 14 identical sequence positions (indicated by *) with 8.434% identity were observed (Fig. 1). In addition, 7 sequence positions of selected eukaryotic RNases (indicated by:) showed conservation with strong similarity score of > 0.5 in Gonnet PAM 250 matrix while 3 sequence positions (indicated by.) showed a conservation between groups of weakly similarities scoring < 0.5. Majority of active sites of these ribonucleases are laying on identical amino acid sequence positions. The hydrophobic properties of protein sequences of selected antitumor ribonuclease enzymes have markedly observed in the N-terminal segments of the query sequences (Fig. 1).

Phylogenetic tree of 14 protein sequences of variants of eukaryotic ribonucleas was then developed using an online bioinformatics tool as indicated in the Methods part. Figure 2 is a phylogram that shows the evolutionary relationship of various monophyletic groups of ribonucleases. The protein sequences of eukaryotic RNases of genus *Lithobates* frogs showed similar recent common ancestor (deep and light purple colored) while protein sequences of RNASO_LITCT from oocytes of *Lithobates catesbeianus* frog (light purple colored) is different from both monophyletic RNase groups of the same frog species (*Lithobates pipiens*). Angiogenin ribonucleases of *Homo sapiens* and bovine *Bos taurus* are from the most recent common ancestor. Similarly, protein sequences of pancreatic RNases of bovine and humans, and seminal RNase of bovine *Bos taurus* also share similar common ancestor. However, the protein sequences of eosinophil cationic protein (ECP) RNase of *Homo sapiens* is completely different from other clades of phylogram and it doesn't show any significant substitution protein sequences from its ancestor.

In addition, the 3D structures of some eukaryotic ribonucleases were fetched from protein data bank (PDB, EMBL-EBI) using a PyMOL™ 2.4.1 (Incentive Product© Schrodinger, LLC) software. Active site amino acid residues of each eukaryotic ribonuclease, their polar interactions and active water molecules were indicated within 5-Angstroms resolution of pre-existed ligands (Fig. 3). Overall similarities of active sites of eukaryotic ribonucleases observed in their 3D structures are also well dictated by the existence of identical positions of active sites of their multiple sequence alignment.

Table 1 Description of eukaryotic ribonucleases of articles included in the review

No	Author, YYYY	RNase Name	RNase Source	RNase Superfamily	Population, Intervention, Comparator and Outcomes (PICOs)				Ref
					Cancer cells / patients	Eukaryotic RNase	Placebo / Other	Apoptosis / Improvement	
1	Laccetti et al., 1992	BS-RNase	Bovine seminal vesicles	Pancreatic-type RNase	Cancer cell lines and mice	BS-RNase	Normal/untreated cells / mice	Growth inhibition, anticancer	[11]
2	Laccetti et al., 1994	BS-RNase	Bovine seminal vesicles	Pancreatic-type RNase	Cancer cell lines & mice	BS-RNase	Normal cells and mice	Antitumor and anti-metastatic	[12]
3	Pouckova et al., 1998	BS RNase	Bull seminal vesicle fluid	Pancreatic-type RNase	Athymic nude mice	BS RNase	Untreated nude mice	Antitumor action	[13]
4	Di Liddo et al., 2010	Bovine milk RNase-4	Bovine milk protein	RNase-4	Cancer cell lines	Bovine milk RNase-4	Untreated cells and RNase-A	Cytotoxicity (growth inhibitory effect)	[14]
5	Patutina et al., 2011	RNase A	Bovine pancreas	RNase A	murine models	RNase A	Saline treated mice	Decreased metastasis	[15]
6	Darzynkiewicz et al., 1988	Pannon (P-30 Protien)	Vertebrate tissue extract	Pancreatic ribonuclease A	Cancer cell lines	Pannon (P-30 Protien)	Untreated cells	Apoptosis	[16]
7	Vasandani et al., *1999*	Onconase (Onc®)	Oocytes of Leopard frog (*Rana* pipiens)	pancreatic ribonuclease A (RNase A)	Apparently healthy mice	Onconase	Unexposed apparently healthy mice	Nephrotoxicity	[17]
8	Lee et al., 2000	Onconase (Onc®)	Amphibian oocytes	Pancreatic ribonuclease A	Cancer cell lines and mice	Onconase (Onc®)	Untreated cells / mice	Antitumor efficacy and lower TIFP	[18]
9	Vogelzang et al.,2001	Ranpirnase (Onc®)	Oocytes of Leopard frog (Ran pipiens)	pancreatic ribonuclease A (RNase A)	Patients With metastasis	Ranpirnase (Onc®)	-	- Survival, - progression time - Toxicities	[19]
10	Magnitsky et al., 2006	Ranpirnase (Onc®)	Eggs & early embryo of Leopard frog *R. pipiens*	Pancreatic ribonuclease A	Cancer cells and nude mice	Ranpirnase (Onc®)	Untreated cells / mice	Apotosis	[20]
11	Lee et al., 2007	Ranpirnase (Onc®)	Oocytes of Northern Leopard *Rana pipiens*	Pancreatic ribonuclease A	Cancer cells and nude mice	Ranpirnase (Onc®)	Normal/untreated cells / mice	Antitumor efficacy	[21]
12	Mikulski et al., 2002	Ranpirnase (Onc®)	Eggs and early embryos of Leopard frog *R. pipiens*	Pancreatic ribonuclease A (RNase A)	Patients with metastasis	Ranpirnase (Onc®)	-	- Survival - Tumor responses - progression Time	[22]
13	Smolewski et al., 2014	Onconase (Onc®) and r-amphinase	Oocytes of Leopard frog (*Rana pipiens*)	pancreatic ribonuclease A (RNase A)	peripheral blood mononuclear/ Cancer cells	Onconase and r-amphinase	Untreated tumor and healthy lymphocytic cells	Cytotoxicity and apoptosis	[23]
14	Ardelt et al., 2007	Amphinase	Oocytes of Northern Leopard (*R. pipiens)*	Pancreatic ribonuclease A	Cancer cell lines	Amph Variants	Alkylated Amph-2	Apoptosis	[24]
No	**Author, YYYY**	**RNase Name**	**RNase Source**	**RNase Superfamily**	**Population, Intervention, Comparator and Outcomes (PICOs)**				**Ref**
					Cancer cells / patients	**Eukaryotic RNase**	**Placebo / Other**	**Apoptosis / Improvement**	
15	Liao et al.,2000	RC-RNase1, 2,3,4,5,6, L1	Bull frog oocyst (*R. catesbeiana*)	Pancreatic ribonuclease A	Cancer cell lines	RC-RNase-1, 2,3,4,5,6, L1	RNase A treated cells	Antitumor efficacy	[25]
16	Wang et al., 2015	Rdchonc RNase	*Rana chensinensis changbaishanensis*	pancreatic ribonuclease A (RNase A)	Cancer cell lines	Rdchonc RNase	Untreated control cells	Antiproliferative, apoptosis and anti-invasion	[26]

Table 1 (continued)

No	Author, YYYY	RNase Name	RNase Source	RNase Superfamily	Population, Intervention, Comparator and Outcomes (PICOs)				Ref
					Cancer cells / patients	Eukaryotic RNase	Placebo / Other	Apoptosis / Improvement	
17	Griffiths et al., 1997	hCG 18 K RNase	Human urinary chorionic gonadotropin	RNase A	Cancer cell lines	hCG 18 K RNase	Untreated cells	Antineoplastic Antiproliferative	[27]
18	Maeda et al., 2002	RNase 3 (ECP)	Large granules of mature peripheral blood eosinophils	pancreatic type RNase	Cancer cell lines	RNase 3 (ECP)	Untreated cells	Growth inhibition	[28]
19	Castro et al., 2011	PE5	Human pancreatic RNase	HP RNase	Cancer cell lines	PE5	Untreated cells	Apoptosis	[29]
20	Zhang et al., 2010	RNLs30 RNase	Fresh fruiting bodies of the edible mushroom *Lyophyllum shimeiji*	Mushroom RNase family	Cancer cell lines	*Lyophyllum shimeiji* RNase	Untreated control cells	Antiproliferative and antitumor	[30]
21	Joceph et al., 2020	Lp16-PSP (Latcripin-16)	Mushroom *Lentinula edodes* C91-3	YjgF/YER057c/UK114	Cancer cell lines	Lp16-PSP (Latcripin-16)	Normal cells	Anticancer and antiproliferative	[31]
22	Kumar et al., 2013	*A. niger* RNase (ACTBIND)	*Aspergillus niger*	-	Cancer cell lines	*A. niger* RNase	Normal cells	Anticancer, antiinvasiveness	[32]
23	Skvor et al., 2006	Wheat leaf RNase (WLN-RNase)	Wheat leaf	Plant ribonuclease	Cancer cells and athymic nude mice	Wheat leaf RNase (WLN-RNase)	RNase A, BS-RNase, Onc treated cells / PBS treated mice	Antiproliferative and antitumor	[33]
24	Fang et al., 2012	MC2 RNase	Dietary bitter gourd (*Momordica charantia)*	MC2 RNase	Cancer cells and nude mice	MC2 RNase	Untreated cells and nude mice	Antiproliferation, tumor growth	[34]

Onc® Onconase®, *WLN* Wheat leaf neutral, *ECP* Eosinophilic cationic protein, *hCG* Human urinary chorionic gonadotropin

Table 2 UniProtKB search results of anticancer eukaryotic ribonucleases

No	Entry name	Protein names	Accession No	Gene names	Length	Active site position		Annotation score (1–5)	Organism
						Proton Acceptor	Proton Donor		
1	RNP30_LITPI	P-30 Protein (Onconase)	EC 3.1.27	-	104			4	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
2	Q8UVX5_LITPI	Onconase variant rpr	-	rpr	127	-	-	-	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
3	Q9I8V8_LITPI	Onconase variant rapLR1	-	-	127	-	-	-	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
4	RNASO_LITCT	Oocytes ribonuclease (RC-RNase) (Sialic acid-binding lectin)	EC 3.1.27	RCR	133	32	125	5	*Lithobates catesbeianus* (American bull frog) (*Rana catesbeiana*)
5	AMPS1_LITPI	Amphinase-1	EC 3.1.27	-	114	15	107	4	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
6	AMPS2_LITPI	Amphinase-2	EC 3.1.27	-	114	15	107	4	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
7	AMPS3_LITPI	Amphinase-3	EC 3.1.27	-	114	15	107	4	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
8	AMPS4_LITPI	Amphinase-4	EC 3.1.27	-	114	15	107	4	*Lithobates pipiens* (Northern leopard frog) (*Rana pipiens*)
9	RNAS1_BOVIN	Ribonuclease pancreatic (RNase A)	EC 4.6.1.18	RNASE1 RNS1	150	38	145	5	*Bos taurus* (Bovine)
10	RNS_BOVIN	Seminal ribonuclease (S-RNase)	EC 4.6.1.18	SRN	150	38	145	5	*Bos taurus* (Bovine)
11	ANG1_BOVIN	Angiogenin-1	EC 3.1.27	ANG1 ANG	148	37	139	5	*Bos taurus* (Bovine)
12	ANGI_HUMAN	Angiogenin (RNase 5)	EC 3.1.27	ANG RNASE5	147	37	138	5	*Homo sapiens* (Human)
13	RNAS1_HUMAN	Ribonuclease pancreatic (HP-RNase)	EC 4.6.1.18	RNASE1 RIB1 RNS1	156	40	147	5	*Homo sapiens* (Human)
14	ECP_HUMAN	Eosinophil cationic protein (ECP)	EC 3.1.27	RNASE3 ECP RNS3	160	42	155	5	*Homo sapiens* (Human)
15	Q6FHX6_HUMAN	Flap endonuclease 1 (FEN-1)	EC 3.1	FEN1 hCG_40848	380	-	-	4	*Homo sapiens* (Human)
16	RNMC_MOMCH	Ribonuclease MC (RNase MC)	EC 4.6.1.19	-	191	34	85	2	*Momordica charantia* (Bitter gourd) (Balsam pear)
17	V5UTC6_LENED	Latcripin-16	Latcripin-16	-	131	-	-	1	*Lentinula edodes* (Shiitake mushroom) (*Lentinus edodes*)
18	F8WSJ0_LYOSH	Ribonuclease T(2) (Fragment)	EC 4.6.1.19	RNLs30	310	-	-	2	*Lyophyllum shimeji* (Hon-shimeji) (*Tricholoma shimeji*)
19	G3XZU9_ASPNA	Ribonuclease	EC 3.1.26.4	ASPNIDRAFT_209236	352	-	-	2	*Aspergillus niger* (strain ATCC 1015)
20	A0A2U7NFE7_WHEAT	Dicer-like protein (Fragment)	Dicer-like protein	dcl4	1392	-	-	2	*Triticum aestivum* (Wheat)

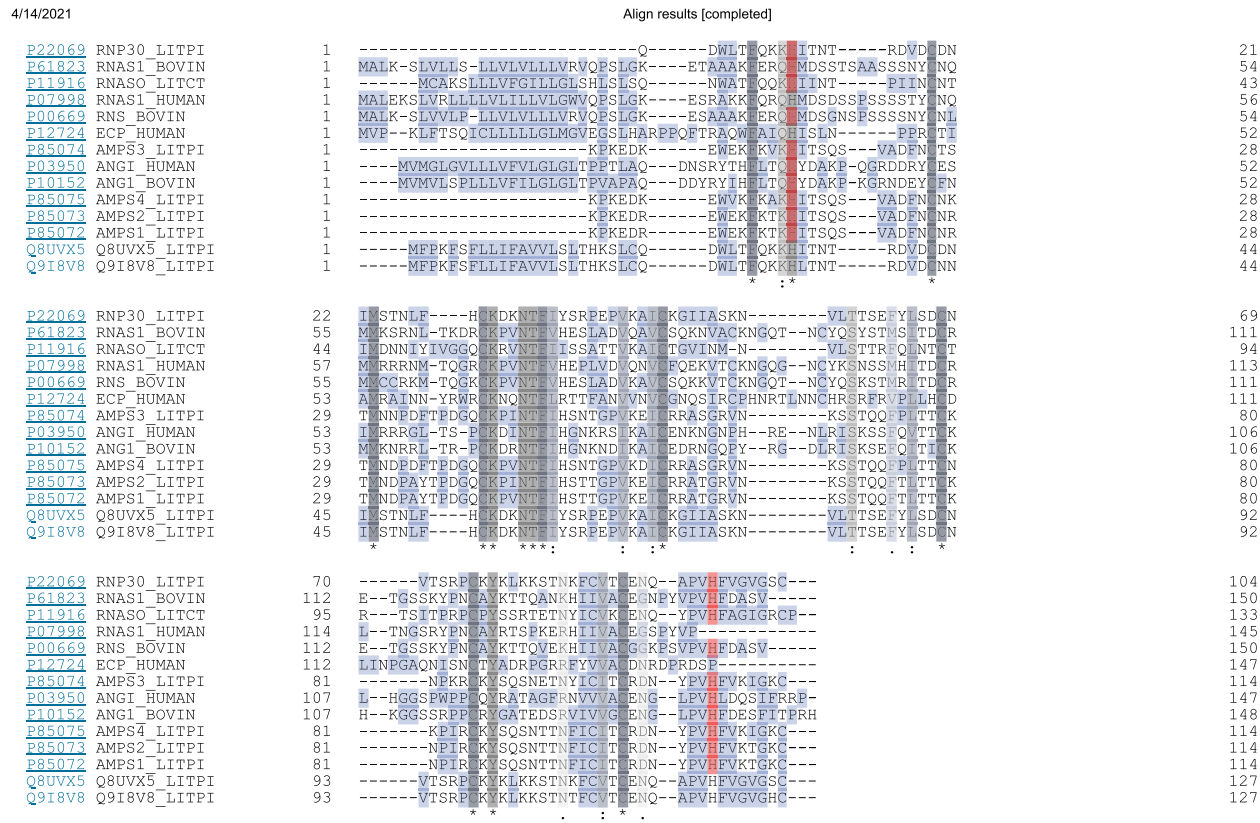

Fig. 1 Active site annotations and amino acid properties of multiple sequence aligned protein sequences of eukaryotic ribonucleases. An asterisk (*) indicates positions which have a single and fully conserved residue, a colon (:) indicates conservation between groups of strongly similar properties—scoring > 0.5 in the Gonnet PAM 250 matrix, a period (.) indicates conservation between groups of weakly similar properties—scoring = < 0.5 in the Gonnet PAM 250 matrix. Red color shaded – active sites of protein sequences, Gray color shaded – identical positions of protein sequence and Blue color shaded – hydrophobic positions of protein sequences

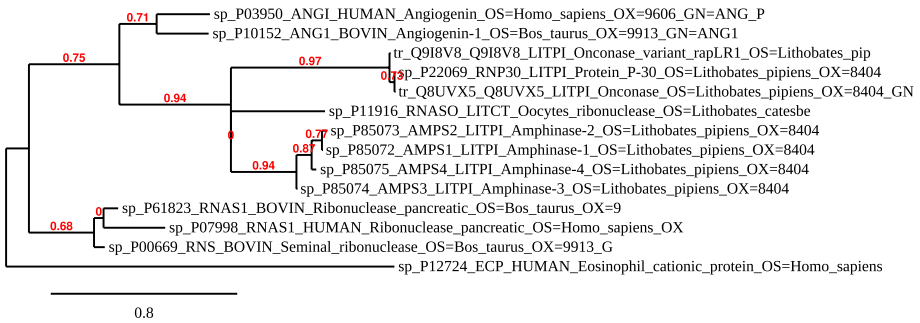

Fig. 2 Phylogenetic tree of 14 protein coding sequences of eukaryotic ribonucleases. A scale bar (0.8) indicates the genetic distance of protein sequences of eukaryotic RNases

In vitro antiproliferative and antimetastasis activity of eukaryotic RNases

Rapid cell proliferation, loss of differentiation and adhesion, and progression of metastasis are some of the features of tumor cells growth [35]. On the other hand, eukaryotic ribonucleases exhibit antiproliferative effect on various types of tumor cell lines and these are now considered a novel class of cancer chemotherapeutic agents [36]. Approximate total cell counts of RNase treated and untreated cellular population were extracted from line graphs of original primary articles

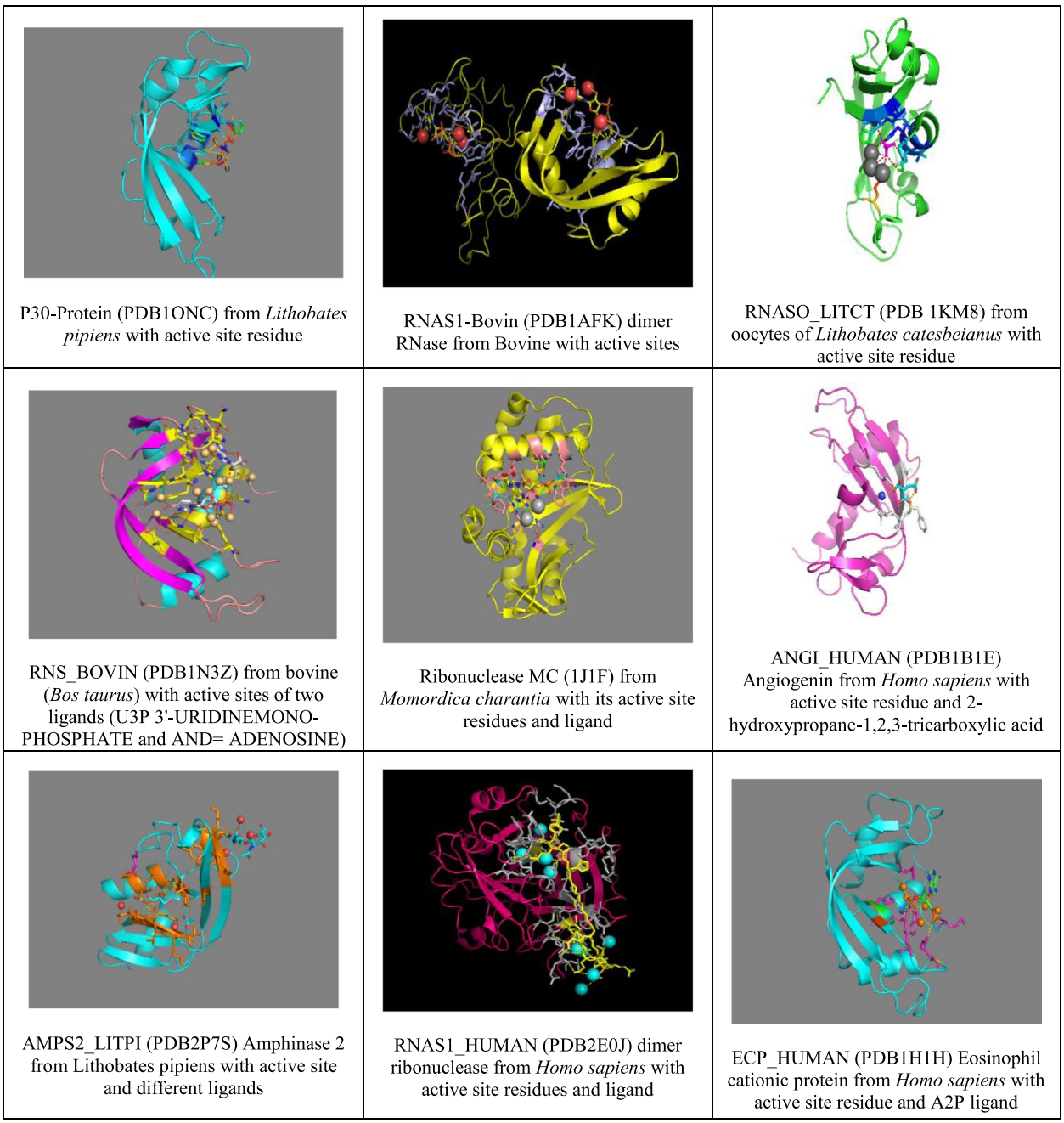

Fig. 3 3D structures of active site residues of certain eukaryotic ribonucleases in relation to preexisted ligands. Sphere in each active site of ribonuclease shows a polar interaction of ligand with water molecules which are considered as active water molecules

and analyzed for tumor cell population in a specified period of incubation (Table 3).

Cell lines of human promyelocytic leukemia (HL-60), human monocytic leukemia (U-937), Jurkat T-cell leukemia, Fischer rat normal thyroid cells (FRTL-5), Fischer rat thyroid tumor cells (TK-6), TK-6 derived lung metastasis (MPTK-6) and drug resistant ovarian cancer cells (NCI/ADR-RES) were treated with increasing concentrations of different classes of RNases (Table 1). A bovine seminal RNase (BS-RNase) treated Fischer rat normal thyroid cells (FRTL-5) showed almost comparable cell population to its control [11] while other RNases such as amphinase [24], P-30 protein [16], PE5 [29] and

Table 3 Total cell number of untreated and RNase treated cell lines over a certain period of incubation

S/N	Author, YYYY	RNase	Experiment		Total cell number* ($\times 10^5$ cells/ml)				Cell Growth (Proliferation)
			Cell lines (Description)	Concentration	0 h	24 h	48 h	72 h	
1	**Ardelt et al., 2007** [24]	Amphinase (Amph)	HL-60 (Human promyelocytic leukemia) U-937 (Human monocytic leukemia) Jurkat cells (T-cell leukemia)	Control	2.0	4.0	10.0	22.0	↓
				Amph, 1 µg/ml	2.0	4.0	5.0	7.0	
				Amph, 5 µg/ml	2.0	3.0	2.5	3.0	
				Amph,10 µg/ml	2.0	2.0	2.5	2.0	
2	**Darzynkiewicz et al., 1988** [16]	P-30 protein (Pannon)	HL-60 (Human promyelocytic leukemia)	Control	2.0	4.5	8.0	17.0	↓
				P-30, 10 µg/ml	2.0	3.0	3.5	5.5	
				P-30, 20 µg/ml	2.0	2.9	2.9	2.0	
3	**Laccetti et al., 1992** [11]	BS-RNase	FRTL-5 (Fischer rat normal thyroid cells)	Control	0.75	5.0	10	10.5	↓↑
				BS-RNase, 5 µg/ml	0.75	5.0	9.5	10.3	
				BS-RNase, 10 µg/ml	0.75	4.5	9.5	10.1	
				BS-RNase, 50 µg/ml	0.75	4.5	9.0	10.0	
			TK-6 (Fischer rat thyroid tumor cells)	Control	0.75	3.5	11.0	13.0	↓
				BS-RNase, 5 µg/ml	0.75	3.5	10.5	12.5	
				BS-RNase, 10 µg/ml	0.75	2.5	10.0	12.0	
				BS-RNase, 50 µg/ml	0.75	2.0	8.0	10.5	
			MPTK-6 (Fischer rat lung metastasis of TK-6 cells)	Control	0.75	4.0	10.5	12.5	↓
				BS-RNase, 5 µg/ml	0.75	2.5	7.5	9.0	
				BS-RNase, 10 µg/ml	0.75	2.0	5.0	8.0	
				BS-RNase, 50 µg/ml	0.75	1.5	3.0	6.0	
4	**Castro et al., 2011** [29]	PE5	NCI/ADR-RES (Drug resistant ovarian cancer cells)	Control	0.07	0.07	0.07	0.07	↓
				PE5, 2 µM	0.07	0.06	0.06	0.05	
				PE5, 14 µM	0.07	0.06	0.05	0.03	
				PE5, 35 µM	0.07	0.06	0.04	0.01	
		Onconase (Onc®)	NCI/ADR-RES (Drug resistant ovarian cancer cells)	Control	0.07	0.07	0.06	0.06	↓
				Onc, 2 µM	0.07	0.06	0.05	0.04	
				Onc, 5 µM	0.07	0.06	0.05	0.04	

*All values of total cell number are approximate value and extracted from each respective reference. ↓ Down arrows indicate the decreasing pattern ofcell proliferation. ↓↑ balanced cell proliferation i.e no difference between treated and untreated groups

Onconase [29, 37, 38] treated cancerous cells showed decreased cell proliferation.

Cell growth inhibition of these ribonucleases was entirely dose and time dependent. As the incubation period and RNase doses increased, the cell growth inhibition increased while cell viability decreased. Brief exposure of BS-RNase to lung metastasis of MPTK-6 cells revealed higher antimetastasis activity while longer period of BS-RNase treatment of the same matastastic cells showed lower antimetastasis activity [11]. This also evidenced by very low cell viability (< 1%) in brief exposure of murine Lewis lung metastasis cells to BS-RNase [12]. Regardless of the concentration of RNases, amphinase [24] and Lp16-PSP [31] RNases treated leukemic cell lines showed much lower viable cells compared to their matched controls (Table 4).

In vitro cytotoxicity of eukaryotic RNases

The half – maximal inhibitory concentration (IC_{50}) of eukaryotic RNases was employed to determine the selective antitumor activity of eukaryotic RNases of some articles included in this review (Fig. 4). A human pancreatic ribonuclease (PE5) treated cancerous and non-cancerous cells demonstrated typical selective cytotoxicity. The highest half – maximal inhibitory concentration of PE5 RNase was found on treated normal human fibroblast cells (N1) ($IC_{50} = 19.5 \pm 1.4$ µM) than cancerous cells including cervical cancer cells (IC50 = 8.2 ± 0.6 µM) and drug resistant ovarian cancer cells ($IC_{50} = 6.9 \pm 0.8$ µM) [29].

In contrary, the same study by Castro *et. al.,* (2011) compared the selective cytotoxicity of PE5 with Onconase, the most known ribonuclease drug [29]. It showed almost similar IC_{50} value of 0.8 ± 0.1 µM, 1.1 ± 0.1 µM

Table 4 Percent cell viability of RNase treated and untreated cell lines

S/N	Author, YYYY	RNase	Experiment		Viable cells (%)* (primary inoculation of < 5.5×10^5 cells/well)				Overall cell viability
			Cell lines (Description)	Concentration	0 h	24 h	48 h	72 h	
1	**Laccetti et al., 1994** [12]	BS-RNase	3LL (Murine Lewis lung metastasis cells)	Control	100	100	100		↓↓↓
				BS-RNase, 0.1 µg/ml	100	95	25		
				BS-RNase, 1.0 µg/ml	100	50	5		
				BS-RNase, 10 µg/ml	100	20	0		
2	**Darzynkiewicz et al., 1988** [16]	P-30 protein (Pannon)	HL-60 (Human promyelocytic leukemia)	Control	100	100	99	99	↓
				P-30, 10 µl/ml	100	90	85	70	
				P-30, 20 µl/ml	100	85	66	48	
3	**Ardelt et al., 2007** [24]	Amphinase (Amph)	HL-60 (Human promyelocytic leukemia) U-937 (Human monocytic leukemia) Jurkat cells (T-cell leukemia)	Control			100	99	↓↓
				Amph, 1 µg/ml			80	55	
				Amph, 5 µg/ml			80	40	
				Amph,10 µg/ml			70	10	
4	**Fang et al., 2012** [34]	RNase MC2	HepG2 (human liver cancer cell)	Control	100				↓
				MC2, 15 µM	100	83	65		
				MC2, 25 µM	100	75	55		
				MC2, 60 µM	100	55	45		
5	**Joseph et al., 2020** [31]	Lp16-PSP RNase	HL-60 (Human promyelocytic leukemia)	Control		100	100		↓
				Lp16-PSP, 50 µg/ml		70	55		
				Lp16-PSP, 100 µg/ml		50	25		
				Lp16-PSP, 150 µg/ml		35	20		
				Lp16-PSP, 200 µg/ml		25	15		

*Appropriate cell densities were initially seeded for each experimental cell model. ↓ Down arrows indicate the decreasing pattern of viability

and 1.0 ± 0.2 µM on cervical cancer, drug resistant ovarian cancer and normal human fibroblast cells respectively. Bovine seminal RNase [12] and RNase from mushroom *L. shimeiji* [30] showed lower IC_{50} towards metastasis-derived Lewis lung carcinoma ($IC_{50} = 0.07 \pm 0.0$ µM) and human liver cancers ($IC_{50} = 6.2 \pm 0.0$ µM) cells respectively.

In vivo antitumor and antimetastasis effect of eukaryotic RNases

Together with other in vivo parameters, tumor volume enabled researchers to determine the antitumor efficacy of eukaryotic RNase in vivo models. In this review, only 25% (6/24) of the total included RNase studies were further assessed tumor volumes of experimental animal models (Table 5).

Appropriate tumor-bearing animal models were selected for each interventional ribonucleases. Then, these animals were xenografted with appropriate cancerous cells subcutaneously. Xenografted tumors were allowed to reach to appropriate tumor volume before any experiment carried out. Treatment groups of animal models were treated with various concentrations of interventional RNases while control groups of animals were injected with PBS/Buffer solution. Modes of administration of intervention or placebo were varied from study to study where intraperitoneal (*i.p.*), inratumoral (*i.t.*), intravenous (*i.v.*) and subcutaneous injections were the most commonly used. Tumor volume of xenografted animals was measured at different days of interval from the 1st day to 23rd day.

Ribonucleases treated xenografted animals showed lower tumor volumes compared to matched control animals (Table 5). For instance, HepG2 tumor-bearing BALB/c nude mice were intraperitoneally injected with a 2 mg/kg of MC2 RNase on every other day [34]. Tumor volume of HepG2 tumor – xenografted animals was measured in mm^3 for several days. The MC2 RNase treated animals showed diminished tumor volume compared to phosphate buffer solution (PBS) injected animals. Higher concentration of bovine seminal RNase (12.5 mg/kg) and lower concentration of wheat leaf ribonuclease (100 µg/mouse in seven doses over a period of 20 days) treated tumor – bearing animals showed lower tumor volume compared to their respective controls. Ranpirnase, Onc®, treated A549 NSCLS xenografted [39], DU145 prostate tumor-xenografted [18] and A549

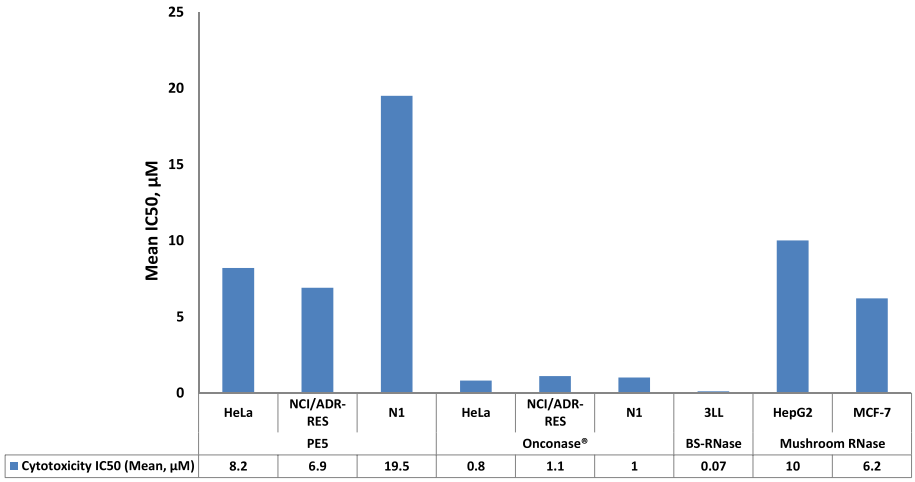

Fig. 4 Cytotoxicity of eukaryotic RNases on cancerous and non-cancerous cell lines. PE5—nuclear directed human pancreatic RNase and BS-RNase – bovine seminal ribonuclease. HeLa – cervical cancer cell line, NCI/ADR-RES – drug resistant ovarian cancer cells, N1 – normal human fibroblast cells, 3LL – Lewis lung metastasis cells, HepG2 – human liver cancer cells and MCF-7 – Breast cancer cells

tumor – bearing athymic nude mice [20] demonstrated diminished tumor volumes compared to the antitumor effect of alkylated onconase in vivo [18].

Furthermore, tumors bearing Fischer rats were induced by highly metastasis cells of Lewis lung cells (3LL) and administered with BS-RNase intraperitoneally. The 10 µg/g and a 20 µg/g body weight of BS-RNase treatment caused about 67% and 92% inhibition respectively against the occurrence of lung metastases compared to untreated animals [12] (Data not shown). Similarly, a study by Patutina and colleagues (2011) examined antimetastasis effect of Pancreatic RNase A towards Lewis lung carcinoma (LLC) and hepatoma A-1 (HA-1) transplanted animals. Hence, H&E stained tissues microscopic examination of lung surface of BS-RNase treated animals revealed a significant decrease in the number of metastases than that of untreated animal [15].

Toxicity and safety of eukaryotic RNases

Table 6 shows safety and toxicity related findings on cell lines, laboratory animals and clinical patients. The overall safety parameters measurements in two clinical trial studies [19, 22] encourage the future application of Ranpirnase in clinical medicine. In phase II clinical trial of ranpirnase, 14 patients with unresectable kidney cells carcinoma were enrolled and adverse events were recorded [19]. In this clinical trial, only a single patient demonstrated a hypersensitivity reaction; so that, the anticancer RNase drug was withdrawn [19]. In recent phase II clinical trial study on patients with unresecteble malignant mesothelioma, 15.2% (16/105) were removed from the study due to renal insufficiency, allergic reaction, proteinuria and other adverse experiences [22]. A nephrotoxicity study by Skvor et al., (2006) documented a reversible proximal tubular toxicity from Onconase® treated apparently healthy mice [33]. In this study, H&E staining of kidney tissues of apparently healthy mice treated with Onconase® demonstrated a moderate multifocal proximal renal tubule necrosis though it was reversed by two weeks after the drug had withdrawn. Ribonucleases MC2, WLN-RNase and BS-RNase treated BALB/c nude mice bearing HepG2 liver cancer, human lymphocytes & athymic nude mice, and 3LL Lewis lung metastasis bearing mice respectively showed promising safe ribonuclease therapy [12, 33, 34]. In these experimental studies, no detectable toxicity to normal tissues, low immunogenicity & embryotoxicity, absence of physical / behavioral changes and absence of allergic symptoms were observed. However, degenerative spermatogenic epithelium has been detected in WLN-RNase treated athymic nude mice [33]. Cell culture-based in vitro toxicity was also determined through half maximal inhibitory concentration (IC_{50}) of RNase Lp16-PSP treated several cell lines [31] where IC_{50} of Lp16-PSP was lower in cancer cells than non-cancerous cells indicating the higher selective cytotoxicity of RNase Lp16-PSP.

Discussion

In spite of major advancements in cancer patient management have been in use, several human cancers, unresectable malignancies in particular continue incurable and consequently, cause serious cancer outcomes. On the other hand, exploring novel anticancer chemotherapeutic agents is undergoing. Antitumor ribonuclease enzymes,

Table 5 Tumor volume of eukaryotic ribonucleases treated xeno-grafted experimental animals

S/N	Author, YYYY	RNase	Experiment			Tumor volume (Mean, mm^3)						Tumor volume
			In vivo model	Drug Administration	Injection Dose	1d	5d	9d	11d	13d	23d	
1	**Fang et al., 2012** [34]	MC2 RNase	BALB/c Nude mice bearing Hep G2 tumor	Intraperitoneal injection (*i.p.*)	Control	10	12	20	30	35	50	↓
					MC2, 2 mg/kg	10	8	7	6	5	1	
2	**Lee et al., 2007** [39]	Ranpirnase (Onc®)	Female athymic NCR-nu/nu nude mice bearing human A549 NSCLC xenograft	Intravenous (*i.v.*) or Intra-peritoneal injection (*i.p.*)	Control	250	275	500	750	950	1750	↓
					ONC, 2.5 mg/kg	250	260	350	650	850	1250	
					ONC, 5 mg/kg	250	250	260	260	550	750	
					ONC, 10 mg/kg	250	250	260	260	250	650	
3	**Lee et al., 2000** [18]	Ranpirnase (Onc®)	DU145 hu prostate tumor xeno-grafted nude mice	Intraperitoneal injection (*i.p.*)	Control, Saline	250	400	550	700	950	1250	↓
					Onconase, alkylated	250	300	400	550	700	1200	
					Onconase, 5 mg/kg	250	100	100	200	300	700	
4	**Magnitsky et al., 2006** [20]	Ranpirnase (Onc®)	A549 tumor bearing Female Athymic NCR-nu/nu nude mice	Subcutaneous	Control, saline	250	250	350	500	550	750	↓
					Cisplatin, 5 mg/kg	250	250	300	350	400	450	
					Onconase, 10 mg/kg	250	250	200	200	250	300	
					Onconase + Cisplatin	250	200	200	150	200	250	
5	**Pouckova et al., 1998** [13]	BS-RNase	Human melanoma xenografted mouse	Intratumoral injection (*i.t.*)	Control	50	70	100	140	300	320	↓
					BS-RNase, 12.5 mg/kg	30	20	20	10	5	4	
		BS-RNase	Mice seminoma xenografted mouse	Intratumoral injection (*i.t.*)	Control	40	50	100	160	180	200	↓
					BS-RNase, 12.5 mg/kg	40	5	0	0	0	0	
6	**Skvor et al.,2006** [40]	WLN-RNase	Athymic human xenograft melanoma nu/nu mice	Intraperitoneal injection (*i.p.*)	Ctrl, PBS,100 µg /mouse	20	40	100	180	250	280	↓
					RNase A,100 µg /mouse	20	40	100	190	240	280	
					WLN-RNase,100 µg/mouse	10	15	30	70	100	120	
					BS-RNase,100 µg /mouse	10	10	15	40	50	70	

d – Days, ↓ – Decreased, Onc – Onconase, BS – Bovine Seminal

Table 6 Safety related findings of RNase treated laboratory models and cancer patients

No	Author, YYYY	RNase	Mol. Wt (kDa)	cell lines / in vivo	Method / Assay	Safety / toxicity
1	**Mikulski et al., 2002** [22]	Ranpirnase	12	Phase II clinical trial on patients with unresectable Malignant Mesothelioma	Safety measurements (frequency of adverse events using physical, vital signs and laboratory evaluations)	- Sixteen (15.2%) of 105 patients were removed from the study because of adverse experiences (renal insufficiency, allergic reaction, proteinuria)
2	**Vogelzang et al., 2001** [19]	Ranpirnase (Onc®)	12	Phase II clinical trial on patients with unresectable kidney cell carcinoma	Physical examinations, lab. analysis and adverse events of 14 patients were performed / recorded weekly	- No higher level toxicities occurred - 5 patients with increased serum creatinine - 1 patient with 4 + proteinuria - Drug withheld in 1 hypersensitive patient
3	**Fang et al., 2012** [34]	RNase MC2	14	BALB/c Nude mice bearing Hep G2 Liver cancer	In vivo: tumor dimension, tumor volume, body weight, tissue Immunohistochemical staining	- No detectable toxicity - No effects on body weight - No toxicity to normal tissues
4	**Skvor et al., 2006** [33]	WLN-RNase	27	human lymphocyte and athymic nude mice	Immunosuppressive activity and Spermatogenic toxicity analysis	- Low immunogenicity & embryotoxicity - degenerative spermatogenic epithelium - No allergic symptoms observed
5	**Vasandani et al., 1999** [17]	Onconase	12	Apparently healthy mice	Kidneys examination using H&E tissue staining	- Proximal tubular toxicity was reversible after two weeks of treatment withdrawn
6	**Laccetti et al., 1994** [12]	BS-RNase	14	- Fischer rat, - 3LL Lewis Lung Carcinoma - metastasis bearing mice	Heamatological tests, Physical & behavioral measurements, tumor & lung size measurement and H&E tissue staining	- No anemia & leukocytosis in treated tumor bearing animals - No physical & behavioral changes in treated healthy animals - No changes in hematologic tests in treated healthy animals
7	**Joseph et al., 2020** [31]	Lp16-PSP	32	HeLa, HepG2, HL-60, HCT-15, SGC-7901, SKOV-3, HaCaT	Cell Counting Kit-8 (CCK-8)	- Higher selective cytoxicity i.e. IC_{50} of Lp16-PSP was lower in cancer cells than non-cancerous cells

a family of small (10 – 28 kDa) basic proteins, are among these researched potential chemotherapeutic agents [41]. Based on the protein sequences, RNases are classified in to different superfamilies [42] including RNase A, H, L, P, E, G, PH, T, I, II and others. Ribonucleases are common ribonucleolytic hydrolases present in cellular entities primarily for RNA processing and maturation [43]. Broadly, ribonucleases are obtained from eukaryotic origin [2, 12, 24, 29, 34, 44] and prokaryotic organisms [45–54]. The selective cytotoxicity of some ribonucleases towards cancerous cells [23, 55, 56] makes RNase a promising alternative chemotherapy in the future clinical patient management. Eukaryotic and prokaryotic origins of RNases in combinations of known anticancer drugs [57–61] and potent anti-malarial drug, dihydroartemisinin, [62] also showed synergistic anticancer effect on in vitro and in vivo studies.

Data retrieval, analysis and presentation of eukaryotic ribonucleases were the main objective of this review. The multiple sequence alignment of protein sequence data of eukaryotic ribonucleases obtained from UniProt knowledgebase showed similar active site positions, 14 identical sequence positions and higher proportion of hydrophobic segments. The higher proportion of hydrophobicity helps the ribonucleases to interact easily with tumor cell and endosomal membranes. Some evidences including [63] showed the absence of hydrophobic segments in the aminoacid sequences of non-cytotoxic ribonucleases. Furthermore, the hydrophobic properties of cytotoxic ribonucleases are essential for lipid – protein interaction and cytotoxicity mechanism [63], and biological energy transduction [64]. On the other hand, increased hydrophobicity of protein sequences of various enzymes may cause loss of their conformational specificity [65].

Twenty four articles on different eukaryotic RNases were selected to explore anticancer potential of eukaryotic RNases. Anticancer potential of these RNases was investigated through cytostatic & cytotoxic parameters of treated cell lines, tumor weight and volume of tumor induced laboratory animals, and survival rates & time to progression of cancer patients under a clinical trial studies. In this review, MTT, TBE, flow cytometer or fluorescent microscopic analysis of treated and untreated cell lines were techniques that most researchers employed to determine the cytotoxicity or cell growth inhibition capacity of eukaryotic ribonucleases. Ribonucleases from oocytes of *Rana pipiens* and other species of genus *Rana* frogs were the most extensively investigated RNase superfamily. Among these amphibian ribonucleases, ranpirnase is a novel RNA targeting drug where degradation of tRNA is considered the main mechanism of its cytotoxicity [66, 67]. Moreover, up-regulation of proapoptotic proteins, mitochondrial transmembrane potential interference, targeting families of microRNAs and antioxidant activity of ranpirnase can also be important elements of its cytotoxic capability towards various cancerous cells [37, 68].

Another clinically potential eukaryotic ribonuclease from amphibians is amphinase, which is obtained from oocytes of Northern leopard frogs (*Rana pipiens*). The cumulative data on amphinase demonstrated marked cell growth inhibition of lymphoid malignancies and other cell lines in amphinase treated cancer cells compared to untreated control cells. Previous reviews concluded that Onconase® and amphinase ribonuclease are able to enter the cellular entities and that target therein is RNA destruction which manifests by observed cytotoxicity and cytotstatic effects [37, 69]. Its fusion with a transforming growth factor-α (TGF-α) protein also exhibited more significant cytotoxicity on high epidermal growth factor receptor (EGFR) expressing tumor cells [70].

In vitro and in vivo preclinical studies of other antitumor eukaryotic ribonucleases (BS RNase, MC2, wheat leaf neutral ribonuclease, Lp16-PSP) open encouraging opportunities to develop safe and effective anticancer drugs. Eukaryotic ribonucleases possess capability to selectively kill cancer cells, minimize metastasis ability of cancer cells & reduce in vivo tumor volume, and lower immunogenicity and toxicities. Unlike DNA-targeting anticancer drugs, ribonucleases are non-genotoxic and their RNA degradation allows altering genetic expression at different phases of cell cycle which leads cancer cells to death [71]. Obviously, the currently used anticancer chemotherapeutics strategies are limited due to their genotoxicity of normal cells, tumor cell heterogeneity, target variability and severe side effects.

A decade years back, on May 28, 2008, an abstract form of a confirmatory phase IIIb clinical trial of Onconase® in combination with doxorubicin of malignant mesothelioma was reported. Although Onconase® did not meet a statistical significance for primary endpoint of survival ($p=0.80$), it showed a median survival time of 11.1 months for Onconase® plus doxorubicin treated MMe patients compared to 10.7 months for doxorubicin alone treated patients [72]. In the same clinical trial, a statistical significant improvement of survival among unresectable MMe patients who previously failed for one prior chemotherapy regimens was reported ($p=0.016$) and the median survival time for Onconase® plus doxorubicin treated evaluable patients was 10.5 months compared to 8.7 months for patients who received doxorubicin drug alone. Hence, based on this preliminary report, the Alfacell Corporation continued New Drug Application (NDA) of Onconase® to the U.S. Food and Drug Administration (FDA) though, to the best of our

knowledge, there is no recent update concerning the status of Onconase® drug.

Phase II clinical trial of Onconase® in malignant mesothelioma revealed that Onconase® is clinically active in which the respective median survival time, 1-year and 2-year survival rates of Onconase® are 11.3 months, 46.2% and 34.3% while its correspondences of doxorubicin are 9.1 months, 34.5% and 10.7% [22]. Another phase II clinical trial in patients with metastatic kidney cancer at a dose of 480 μg/m^2/w, Onconase® showed a minimal clinical activity with a median survival time of 16 months (ranging from 2 to 28 months) [19]. These clinical trials were carried out after a safety observations of phase I clinical trial of Onconase® in patients with solid tumors [73]. The maximum tolerated dose was 960 μg/m^2 and the study concluded that Onconase® was well tolerated by majority of patients.

Conclusion and future perspective

Most eukaryotic ribonucleases are at preclinical stages of drug discovery. Their selective cytotoxicity makes them promising candidates of anticancer chemotherapeutics by which genotoxicity of the current anticancer drugs can be relieved. Ranpirnase, Onconase®, achieved encouraging outcomes from different clinical trials particularly in the treatment of unresectable cancers including malignant mesothelioma. However, clinical studies of other ribonucleases of eukaryotic sources are still at their early stages. Hence, further in vivo investigations (i.e. clinical trials) of eukaryotic ribonucleases provide concrete evidences in recruiting alternative anticancer chemotherapeutic agent.

Abbreviations

Amph: Amphinase; ATP: Adenosine Triphosphate; BS RNase: Bovine Seminal RNase; ECP: Eosinophilic Cationic Protein; EGFR: Epidermal Growth Factor Receptor; FDA: Food and Drug Administration; hCG: Human Urinary Chorionic Gonadotrophin; H&E: Hematoxylin and Eosin; IC_{50}: Half-maximal Inhibitory Concentration; *i.p.*: Intraperitoneal; *i.t.*: Inratumoral; *i.v.*: Intravenous; kDa: killo Dalton; Lp16-PSP: Latcripin 16-Perchloric-acid Soluble Protein; MEGA: Molecular Evolutionary Genetics Analysis; MeSH: Medical Subject Heading; MMe: Malignant Mesothelioma; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); NCBI: National Center for Biotechnology; NDA: New Drug Application; NSCLS: Non Small Cell Lung Carcinoma; Onc®: Onconase®; PBS: Phosphate Buffer Solution; PDB: Protein Data Bank; PIR: Protein Information Resource; RNA: Ribonucleic Acid; RNase: Ribonuclease; RI: Ribonuclease Inhibitor; TBE: Trypan Blue Exclusion; TGF-α: Transforming Growth Factor-α; TrEMBL: Translated EMBL; UniProtKB: Universal Protein Knowledge Base; WLN: Wheat Leave Neutral; 3D: Three Dimensional.

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Authors' contributions

YAS conceived the research idea and wrote this review manuscript. The author collected relevant articles and summarized the data obtained. The author read and approved the final manuscript.

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Availability of data and materials

Summaries of information were included in the study in the form of texts, tables and figures. Conclusions were also drawn from these data. Raw data can be accessed from the author up on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

I declare that I have no competing interests.

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