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Review

Ribonucleases of different origins with a wide spectrum of medicinal applications

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ABSTRACT

Ribonucleases (RNases) are a type of nucleases that catalyze the degradation of RNA into smaller components. They exist in a wide range of life forms from prokaryotes to eukaryotes. RNase-controlled RNA degradation is a determining factor in the control of gene expression, maturation and turnover, which are further associated with the progression of cancers and infectious diseases. Over the years, RNases purified from multiple origins have drawn increasing attention from medical scientists due to their remarkable antitumor properties. In this review, we present a brief summary of the representative RNases of fungal, bacterial, plant, and animal origins and outline their potential medicinal value in the treatment of tumor and AIDS. Among them, the most clinically promising RNases are mushroom RNases, Binase and Barnase from bacteria, ginseng RNases, and Onconase from frog (*Rana pipiens*). Fast developing protein engineering of RNases, which display more potent cytotoxic activity on and greater selectivity for malignant cells, has also aroused the interest of researchers. The multiple anti-cancer mechanisms of RNases are also included. To sum up, these inspiring studies unveil a new perspective for RNases as potential therapeutic agents.

Contents

1. Introduction

RNA-degrading enzymes, better known as ribonucleases (abbreviated as RNases), catalyze the degradation of RNA into smaller components [1,2]. With the use of chromatographic techniques, a large number of RNases in prokaryotes and eukaryotes have been purified [3–9]. Knowledge of the activities, sequences and structures of RNases facilitates their classification into endoribonucleases and exoribonucleases. They comprise several sub-classes within the EC 2.7 (phosphorolytic cleavage) and 3.1 (hydrolytic cleavage) classes of enzymes [1,10]. The typical structural characteristics of four representative RNases from the kingdoms of fungi, bacteria, plantae, and animalia are shown in Fig. 1 [11–14].

Some members of this RNase family exhibit angiogenic, neurotoxic, antitumor, or immunosuppressive activities [7,15]. Among the noteworthy activities, RNases such as the paradigm Onconase from frog (*Rana pipiens*), show significant antitumor activity. They have attracted a special interest for they could be used as potential therapeutics on different malignancies [7]. Besides using naturally purified RNases, major efforts have been invested in the development of engineered RNases, which display more potent cytotoxic activity and greater selectivity for malignant cells [5,16–32].

In this article, we focus our attention to cover representative RNases of fungal, bacterial, plant, and animal origins. In particular, we summarize the most recent findings and highlight of previous work in

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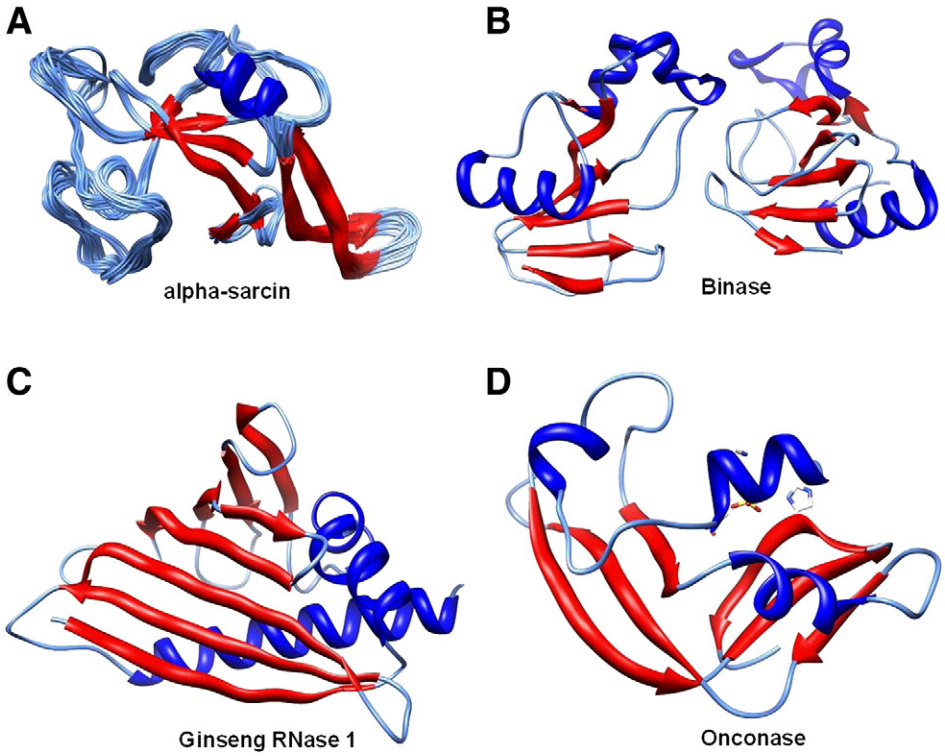

Fig. 1. Stereoscopic views of representative RNases of different kingdoms as fungi (A), bacteria (B), plantae (C), and animalia (D). (A) Crystal structure of α-sarcin from *Aspergillus giganteus* (Protein Data Bank/PDB ID: 1DE3) [62]. (B) Crystal structures of *Bacillus intermedius* RNase (Binase) (PDB ID: 1GOU) [12]. (C) Predictive 3-D structure of ginseng RNase 1 [86] created by the on line Phyre webserver [87]. (D) Structure of an amphibian ribonuclease Onconase (P-30 protein) (PDB ID: 1ONC) [14]. Secondary ribbon structure of helix and strand are demonstrated in blue and red, respectively. Structures were downloaded from the protein data bank with Chimera for visualization [136].

the areas of RNase biochemistry, *in vitro* and *in vivo* antitumor and anti-HIV activities, and molecular mechanisms of RNase-induced cytotoxicity. Reviewing current research on the applications of RNases allows conjectures on their promising medicinal future.

2. Medicinal RNases of different origins

Due to recent progress in RNase research, the prospects of using RNases in the fields of tumor diagnosis and treatment have greatly improved. Here we review structures, functions, mechanisms, and potential applications of a list of natural and engineered RNases. Besides aiming at the antineoplastic activity of RNases, we also focus on their activity in inhibiting human immunodeficiency virus (HIV) which is promising for use in treatment of acquired immune deficiency syndrome (AIDS) [33].

2.1. Medicinal RNases from fungi

Mushrooms form a large part of the kingdom of fungi. The medicinal use of mushrooms, such as *Cordyceps sinensis*, *C. militaris*, *Coriolus versicolor*, *Ganoderma lucidum*, *Grifola frondosa*, and *Hericium erinaceus*, has a very long tradition in the Asian countries, whereas their use in the Western hemisphere is steadily increasing [34–36]. Using mushrooms as a source to isolate medicinal compounds and proteins is a meritorious way to find novel antineoplastic drugs which have been extensively studied (Table 1) [37,38]. Here we focused on the isolation of RNases from medicinal and edible mushrooms, as well as their interesting medicinal activities.

A 9.5-kDa peptide manifesting RNase activity was isolated from the mushroom *Agrocybe cylindracea*. It exerted antiproliferative activity on leukemia cell line (M1) and hepatoma cell line (HepG2), with an IC_{50} of 10 and 100 μM, respectively [39]. Its potent activity in the production of nitric oxide, a major effector molecule in cancer prevention, may contribute in part to its antitumor activity [39–41]. Two RNases have been extracted from the mushroom *Calvatia caelata* [42,43]. One is a ubiquitin-like 8-kDa peptide. It inhibited the proliferation of breast cancer cells (IC_{50} 100 nM) [42]. Another RNase is calcaelin which displayed a heat-labile RNase activity of 1.58 U/mg toward yeast tRNA. It exhibited antimitogenic activity toward mouse splenocytes, and reduced the viability of breast cancer cells [43].

An 18-kDa RNase, which demonstrated the highest ribonucleolytic activity (196 U/mg) toward poly C, was purified from the mushroom *Hypsizigus marmoreus*. The RNase inhibited the proliferation of leukemia L1210 cells with an IC_{50} of 60 μM [44]. Another polyU- and polyC-specific RNase was purified from fresh fruiting bodies of the edible mushroom *Lyophyllum shimeji*. This 14.5-kDa protein exhibited cytostatic activity toward liver cancer HepG2 cells (IC_{50} 10 μM) and breast cancer MCF7 cells (IC_{50} 6.2 μM). It inhibited the activity of HIV-1-RT with an IC_{50} of 7.2 μM [45].

RNases of many mushroom species in the *Pleurotus* genus have also been studied. They comprise those of *Pleurotus djamor* [46], *P. eryngii* [47], *P. ostreatus* [48–53], *P. sajor-caju* [54,55], *P. pulmonarius* [56], and *P. tuber-regium* [57]. Some of these RNases contain antitumor activity and/or anti-HIV-RT activity (details in Table 1). For instance, a 15-kDa RNase from *P. djamor* manifested antitumor activity on Hep G2 cells (IC_{50} 3.9 μM) and MCF-7 cells (IC_{50} 3.4 μM) [46]. Furthermore, a 14-kDa RNase from the edible wild mushroom *Russula delica* was reported. It demonstrated activity against the malignant HepG2 and MCF-7 cancer cells but was devoid of anti-HIV-1-RT activity [58]. Unlike *Russula delica* RNase, a 30-kDa RNase from the edible wild mushroom *Thelephora ganbajun* manifested a puissant inhibitory activity toward HIV-1-RT (IC_{50} 0.3 μM) [59].

Since studies on the antitumor mechanism of mushroom RNases are limited, future investigations on this field may facilitate their potential medicinal applications. To this end, a summary of the in-depth study on α-sarcin may shed light on carrying out associated studies on mushroom RNases. Alpha-sarcin is the most prominent member of a