

Review

The role of human ribonuclease A family in health and diseases: A systematic review

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SUMMARY

The ribonuclease A (RNase A) family is one of the best-characterized vertebrate-specific proteins. In humans, eight catalytically active RNases (numbered 1–8) have been identified and have unique tissue distributions. Apart from the digestion of dietary RNA, a broad range of biological actions, including the regulation of intra- or extra-cellular RNA metabolism as well as antiviral, antibacterial, and antifungal activities, neurotoxicity, promotion of cell proliferation, anti-apoptosis, and immunomodulatory abilities, have been recently reported for the members of this family. Based on multiple biological roles, RNases are found to participate in the pathogenic processes of many diseases, such as infection, immune dysfunction, neurodegeneration, cancer, and cardiovascular disorders. This review summarizes the available data on the human RNase A family and illustrates the significant roles of the eight canonical RNases in health and disease, for stimulating further basic research and development of ideas on the potential solutions for disease diagnosis and treatment.

INTRODUCTION

Upon the completion of the human genome project, eight canonical RNase A genes were found on chromosome 14q11.2 (Cho et al., 2005; Goo and Cho, 2013; Sorrentino, 2010). These include RNase 1 (pancreatic RNase), RNase 2 (eosinophil-derived neurotoxin, EDN), RNase 3 (eosinophil cationic protein, ECP), RNase 4, RNase 5 (angiogenin, ANG), RNase 6 (RNase k6), RNase 7, and RNase 8 (Koczera et al., 2016; Sorrentino, 2010). This chromosome location also includes several homologous genes (RNases 9–13), which are related to the RNase A family based on amino acid sequence homology; however, these genes lack one or more elements necessary for enzymatic activity (Goo and Cho, 2013). Collectively, the eight canonical members (RNases 1–8) exhibit 40% sequence identity, whereas RNases 9–13 show only 15–30% identity with the canonical RNases (Cho et al., 2005). The human RNase A superfamily members exhibit diverse expression patterns (Lu et al., 2018), which are summarized in Table 1.

Mature RNases are secretory proteins that share the specific elements of the sequence signature (Figure 1A), a kidney-shaped tertiary structure stabilized by disulfide bonds, and a catalytic center comprising two histidine residues and one lysine residue (Figure 1B) (Cuchillo et al., 2011). As enzymes, these proteins mainly digest single-stranded RNA substrates, such as tRNA, rRNA, or mRNA, by catalyzing the trans-phosphorylation and hydrolysis of the phosphodiester bond (Castro et al., 2021; Cuchillo et al., 2011; Raines, 1998). However, their ribonucleolytic activity varies in magnitude. Compared with RNase 1, RNase 2 and 4 have similar activities, RNase 3, 6, 7, and 8 exhibits 8–100-fold lower activity, whereas RNase 5 has the lowest activity (approximately 10⁻⁵-fold) (Sorrentino, 2010; Wang et al., 2018b; Zhang et al., 2003). Accumulating evidence indicates that these proteins can be internalized into cells through multiple pathways, including membrane receptors-dependent endocytosis or dynamin-independent penetrating (Chao and Raines, 2011; Ferguson and Subramanian, 2018; Yu et al., 2017), and then regulate cellular processes in a ribonucleolytic activity-dependent or -independent manner. The distinct ribonucleolytic activity and various cellular functions of RNases suggest that they play important roles in the human body.

RNase A family members play critical roles in many biological processes, including antiviral, antibacterial, and antifungal activities in innate immunity as well as neurotoxicity, immunomodulation, promotion of cell proliferation, anti-apoptosis, and regulation of intra- or extra-cellular RNA metabolism (Lu et al., 2018; Rosenberg, 2008b). Clinical investigations and research using animal disease models also indicate that they play significant roles in several physiological and pathological conditions. In infectious diseases, all

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Table 1. The characteristics of human RNase A family

RNase	RNase Activity (relative to RNase1)	Net charge ^a	Expression pattern	
			Organs ^b	Cell types ^c
RNase 1	1	+11	Various organs, such as pancreas, lung, stomach, testis and so on	Endothelial cells, acinar cells, oligodendrocyte, enterocytes
RNase 2/EDN	0.65 (Wang et al., 2018b)	+7	Bone marrow	Neutrophils, dendritic cells, monocytes
RNase 3/ECP	4.8×10^{-2} (Wang et al., 2018b)	+13	Bone marrow	Neutrophils
RNase 4	~ 1 (Wang et al., 2018b)	+16	Various organs, such as liver, pancreas, intestine, ovary and so on	Fibroblasts, microglia, macrophages, keratinocytes.
RNase 5/ANG	1×10^{-5} (Sheng and Xu, 2016)	+11	Various organs, such as liver, pancreas, intestine, stomach and so on	Microglia, macrophages, hepatocytes, fibroblasts,
RNase 6/RNase k6	2.5×10^{-2} (Rosenberg and Dyer, 1996)	+8	Various organs, such as lymph node, spleen, gall bladder, urinary bladder and so on	Macrophages, dendritic cells, B cells, plasmacytoid dendritic cells,
RNase 7	0.14 (Zhang et al., 2003)	+16	Esophagus, skin, gall bladder	No data
RNase 8	0.08 (Zhang et al., 2003)	+4	Placenta, lung, spleen and testis	No data
RNase 9	Inactive	–	Testes, epididymis	No data
RNase 10	Inactive	–	Epididymis (Krutskikh et al., 2012)	Germ cells
RNase 11	Inactive	–	Testes	No data
RNase 12	Inactive	–	No data	Neutrophils, basophils, eosinophils
RNase 13	Inactive	–	Testes	No data

^atheoretically calculated.

^bdata from NCBI gene expression (www.ncbi.nlm.nih.gov/gene/).

^cdata from single cell RNA sequencing database (PanglaoDB).

canonical human RNase A family members have been reported to exert antimicrobial effects in a tissue-specific and target-selective manner (Koczera et al., 2016). Furthermore, RNase 2, 3, 5, and 7 participate in regulating allergies or immune diseases of the skin, the respiratory system, and the gut (Lu et al., 2018). In tumors, RNase 5 has been shown to promote tumor growth and metastasis (Sheng and Xu, 2016); however, RNase 1 is considered as candidate therapy for tumors (Castro et al., 2021). In neurological disorders, RNase 2 and 3 are considered risk factors because of their potential neurotoxicity (Singh and Barta, 2011), whereas RNase 4 and 5 are found as neuroprotective factors (Li et al., 2013; Sebastia et al., 2009). In the cardiovascular system, RNase 1 maintains vesicular homeostasis by digesting circulating RNA (Bedenbender and Schmeck, 2020), and RNase 5 is considered to promote endothelial cell proliferation (Kishimoto et al., 2005). In the male reproductive system, RNases 9 and 10 are involved in sperm maturation and male fertility (Cheng et al., 2009; Krutskikh et al., 2012). In this review, we summarized the available information on RNase functions and their underlying mechanisms in different human diseases, to provide a novel perspective in better understanding their physiological and pathological significance and to promote their clinical application (Figure 2).

INFECTIOUS DISEASES

Members of the human RNase A family display a wide spectrum of antimicrobial activities, supporting the hypothesis that they may be involved in host defense. Like other host defense peptides, the primary bactericidal mechanism of these proteins depends on their ability to disrupt bacterial cell walls. This is driven by net charge, amphipathicity, disulfide bonding, and secondary structure of the proteins. In addition to their membrane penetrating capability, RNase A family members can interfere with bacterial attachment and translocate into bacterial cells to inhibit protein and/or DNA synthesis. To host cells, they can initiate signaling pathways that are important in innate immunity and inflammatory responses. Here, we focus on their roles in infectious diseases caused by microorganisms such as bacteria, viruses, fungi, or parasites.

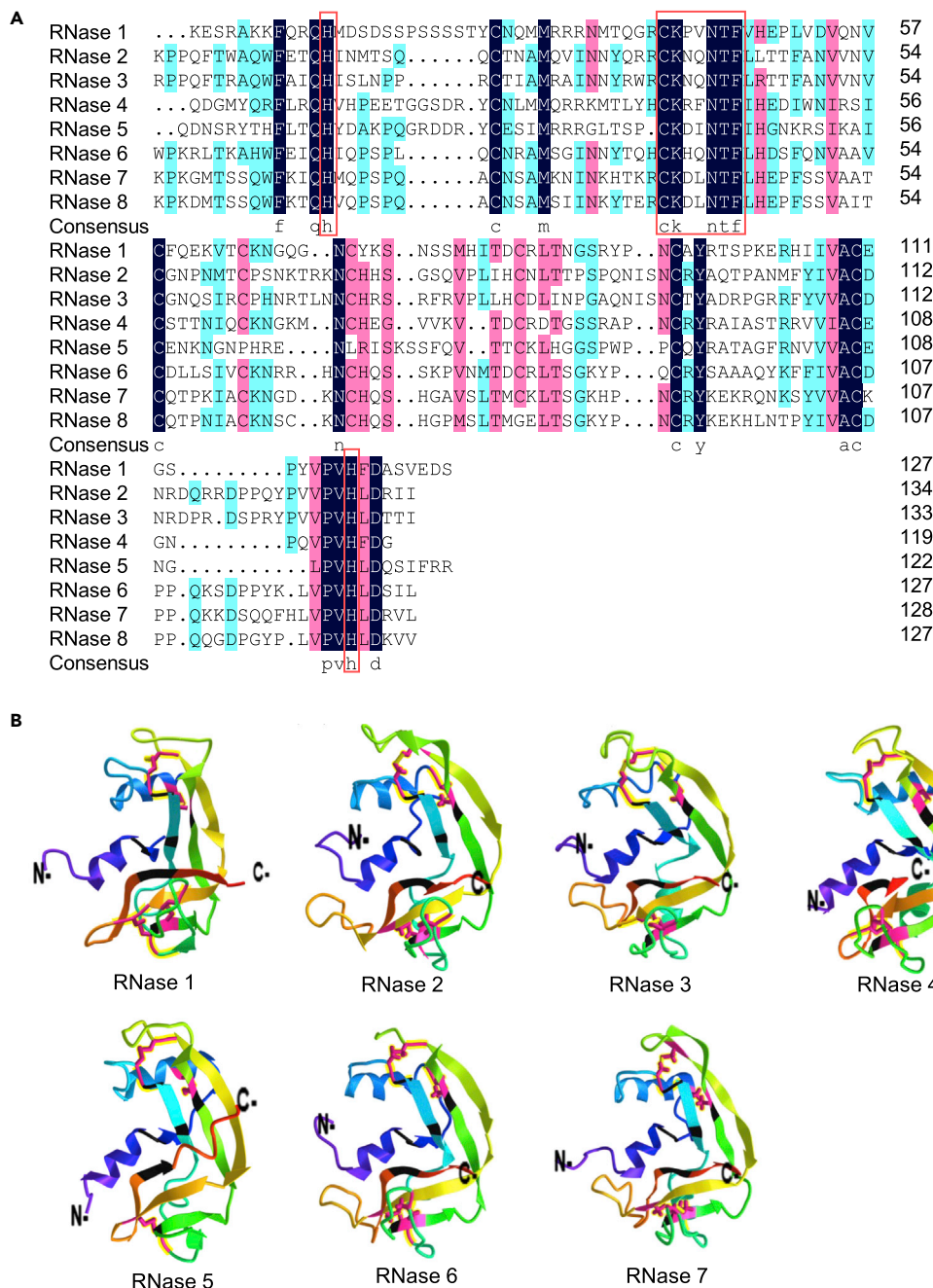


Figure 1. Amino acid sequence alignment and 3-D structures of canonical RNase A family members

(A) The amino acid sequence alignment of RNase 1 (NCBI: NP_937878.1), RNase 2 (NCBI: NP_002925.1), RNase 3 (NCBI: NP_002926.2), RNase 4 (NCBI: NP_001269121.1), RNase 5 (NCBI: NP_001372202.1), RNase 6 (NCBI: NP_005606.1), RNase 7 (NCBI: NP_115961.3), and RNase 8 (NCBI: NP_612204.1). The red box shows the catalytic active sites.

(B) Ribbon model shows 3-D structures of RNase 1 (PDB: 1DZA), RNase 2 (PDB: 1GQV), RNase 3 (PDB: 1QMT), RNase 4 (PDB: 1RNF), RNase 5 (PDB: 1B1I), RNase 6 (PDB: 6MV6), and RNase 7 (PDB: 2HKY). The catalytic active sites are shown in black, and the disulfide bonds are shown in deep pink. (The 3-D structure data of RNase 8 is not available in PDB).

Respiratory tract infection

Infectious diseases of the respiratory tract are mainly caused by viruses or bacteria. Respiratory syncytial virus (RSV) and coronaviruses (CoVs) are single-stranded RNA viruses that can cause highly contagious

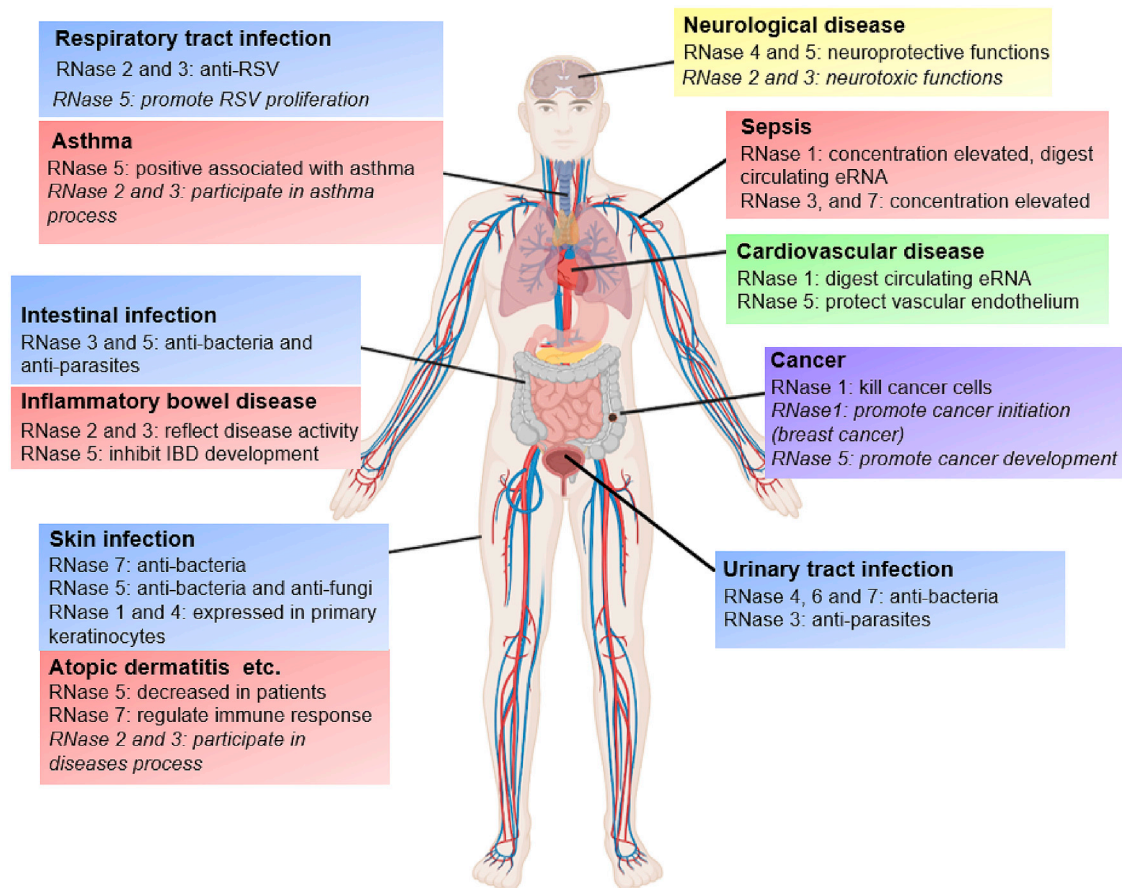


Figure 2. Research advances in the human RNase A family in diseases

Human RNase A family participated in several diseases. They are mainly distributed in the nervous system, respiratory system, cardiovascular system, urinary system, digestive system, and epidermal tissue system. Their functions in diseases are classified into 5 subsets, including infectious disease (blue block), immune dysregulated disease (red block), neurodegenerative disease (yellow block), cardiovascular disease (green block), and cancers (purple block). The beneficial proprieties are shown in normal font, the harmful proprieties are indicated in italics. The graphic is created with the software tool BioRender.

respiratory tract diseases. The ongoing outbreak of the novel coronavirus (2019-nCoV) has spread in most countries worldwide and has become a global public health emergency (Singh et al., 2021). Therefore, there is an urgent need to develop new agents against respiratory tract infections. In recent years, accumulating evidence suggests that airway epithelial cells and neutrophils produce and secrete soluble host defense peptides, including members of the RNase A family, to combat invasive microorganism challenges (Koczera et al., 2016; Rosenberg, 2008b). For example, RNase 2 and 3 are upregulated during RSV infection (Domachowski et al., 1998a, 1998b). Furthermore, RNase 2 levels in serum are increased following RSV-induced bronchiolitis and are used as a predictive marker for recurrent wheezing (Kim et al., 2013). As host defense peptides with nuclease activity increased expression of RNases is understandable as they may directly protect against viruses by penetrating the viral capsid and degrading the viral RNA (Domachowski et al., 1998b). RNase 2 has higher antiviral activity than that of RNase 3 owing to the presence of a specific region at its C-terminal loop L7, which is beneficial for the interaction of the protein with viral capsid in turn promoting protein's entry into the virion (Sikriwal et al., 2012). Consistently, mouse eosinophil-associated ribonuclease 2 (mEAR 2), a homologous protein of human RNase 2, also has antiviral activity against pneumonia virus of mice (PVM), a rodent virus related to RSV (Rosenberg, 2015). There is no evidence that RNases have a direct effect on CoVs; however, RNase 2 is upregulated upon SARS-CoV infection, and it has been proposed as a novel clinical biomarker of COVID-19 (Dosanjh, 2020; Lee et al., 2005). Most recently, increasing levels of eosinophils (the main secretion cells of RNase 2) have been related to a better prognosis for recovery from COVID-19 (Dosanjh, 2020). Besides, comparative analysis of genomes provides that RNase 4 gene expands seven complete and two partial copies in bat, which is a notorious reservoir

host for some of the world's most highly pathogenic virus, including CoVs (Zhang et al., 2013), suggesting that RNase 4 may also play a role in virus resistance. Thus, RNases may offer an alternative method for fighting against viral infections of the respiratory tract.

Viruses hijack the host's cellular machinery to replicate themselves and spread to new cells. As secreted proteins with nuclease activity, RNases can be internalized by infected cells to digest RNAs, thereby playing an important role in regulating antiviral innate immune responses (Wu et al., 2020). In fact, small noncoding RNAs derived from tRNAs, such as tRNA-derived stress-induced RNAs (tiRNAs) and tRNA-derived fragments (tRFs), have been identified and proven to act as functional regulatory molecules in RSV infection (Wang et al., 2013). RSV infection together with other cellular stresses can induce RNase 5-mediated tiRNA and tRF production. Surprisingly, the release of a specific tRF, tRF5-Glu^{CTC}, which targets and suppresses apolipoprotein E receptor 2 (APOER2), can promote RSV replication (Deng et al., 2015). Lu et al. observed that RNase 2 is also involved in cellular tRF production during RSV infection (Lu et al., 2022). However, further studies are warranted for an unambiguous pattern assignment to understand how RNases can shape the cellular RNA population and their role against viral infection.

Besides, RNase 3, 6, and 7, secreted by leukocytes or epithelial cells in airway, are involved in counteracting *Mycobacterium* infection (Lu et al., 2019; Pulido et al., 2013). Pulido et al. reported that RNase 3 and 7 can effectively suppress *Mycobacterium vaccae* at a low micromolar level *in vitro*, with bacterial membrane depolarization and permeabilization activities being the main bactericidal mechanisms (Pulido et al., 2013). Lu et al. found that RNase 3 and 6 not only inhibit *Mycobacterium aurum* (*M. aurum*) growth extracellularly but also help to kill the *M. aurum* located in macrophages by inducing autophagy. By using RNase3-H15A enzymatically inactive variant, it is demonstrated that RNase 3-mediated *M. aurum* killing or autophagy induction is RNase activity independent (Lu et al., 2019). Moreover, Amatngalim et al. found that another respiratory pathogen *Haemophilus influenzae* can significantly increase RNase 7 expression in cultured airway epithelial basal cells (Amatngalim et al., 2015). Although increasing pieces of evidence suggest some RNase A family members may protect airway from infection, further studies are needed to reveal all the members' bactericidal functions in the respiratory system defensive reaction.

Skin infection

The skin is an important surface tissue that is inevitably exposed to various environmental microorganisms and also has the risk to be infected by pathogenic bacteria or fungi. RNase 1, 4, 5, and 7 are highly expressed in human keratinocytes and may thus contribute to cutaneous innate defense (Abtin et al., 2009). Among the four members, RNase 7 is the most studied protein in skin infection, because it was originally isolated from stratum corneum extracts in an attempt to identify and characterize the antimicrobial factors produced by healthy human skin (Harder and Schroder, 2002). Despite the relatively high baseline levels of RNase 7 in keratinocytes, its expression can be further induced by interleukin-1 β (IL-1 β), ultraviolet B radiation, and bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) (Harder and Schroder, 2002; Mohammed et al., 2011). RNase 7 is expressed throughout the human skin, but its expression levels vary across different sites. For example, in a healthy volunteer, RNase 7 was found at 0.93 ng/cm² on the forehead, 2.0 ng/cm² on the hand, and 3.4 ng/cm² on the calf (Köten et al., 2009). RNase 7 exhibits broad-spectrum activity against gram-positive and gram-negative bacteria, such as *S. aureus*, *P. aeruginosa*, and *Enterococcus faecium* (*E. faecium*) as well as against the yeast *Candida albicans* (*C. albicans*) (Köten et al., 2009; Rademacher et al., 2017). Notably, it is also active against multidrug-resistant bacteria such as methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecium* (Harder and Schroder, 2002). The antimicrobial activity of RNase 7 is independent of its ribonuclease activity but is dependent on its positive charge (Becknell and Spencer, 2016). Huang et al. reported that membrane permeabilization by RNase 7 requires four clustered lysine residues located at the flexible coil near the N terminus (Huang et al., 2007). Lin et al. reported that the binding of RNase 7 to *P. aeruginosa* outer membrane protein I (OprI) is a prerequisite for its bactericidal action against *P. aeruginosa*. The activity of RNase 7 against *P. aeruginosa* was blocked by the addition of exogenous OprI or anti-OprI antibodies (Lin et al., 2010). These results indicate that the capacity to bind bacterial cell surface structures is a critical step in the bactericidal action of RNase 7. However, further studies are necessary to decipher the exact bactericidal mechanism(s) of RNase 7 and to assess its strain-dependent differences.

Although RNase 5 expression is lower than that of RNase 7 (only one-fifth) in keratinocytes, RNase 5 was more efficient than RNase7 in killing *C. albicans*, a common fungal pathogen on the skin surface (Abtin

et al., 2009). Unlike its mechanism of action against bacteria, enzymatic activity plays a critical role in the antifungal activity of RNase 5, but its molecular target remains unclear (Abtin et al., 2009). RNase 1 and 4 are also reported to be expressed in primary keratinocytes (Abtin et al., 2009), and recent *in vitro* studies showed that they have potent antibacterial activity (Bender et al., 2021; Kosgey et al., 2020). Taken together, cutaneous expression of RNases may be involved in protecting the skin against infection; however, the functional relevance of RNases in controlling microbial growth at the skin surface needs to be demonstrated conclusively.

Urinary tract infection

Urinary tract infections (UTIs) are some of the most common infections encountered in clinical medicine. Uropathogenic *Escherichia coli* (UPEC) is the causative uropathogen identified in 80% of all UTIs, and other pathogens include *Klebsiella*, *Staphylococcus*, and *Enterococcus*. The parasite *Schistosoma haematobium* also causes considerable urogenital disease, especially in Africa (Lacerda Mariano and Ingersoll, 2020). Recent data suggest that RNase 4 and 7 are abundantly produced by bladder urothelium and the renal collecting duct, and released into the urine (Bender et al., 2021; Spencer et al., 2013). In response to microbes, circulating leukocytes that harbor RNase 3 (eosinophils and neutrophils) and 6 (monocytes and macrophages) exit the bloodstream and cross the urothelium to accumulate in the urine (Becknell et al., 2019). Research on RNases in urinary tract infections has primarily focused on their bactericidal activity.

A series of studies by John David Spencer indicate that RNase 4 and 7 play a role in maintaining human urinary tract sterility (Bender et al., 2021; Spencer et al., 2013). Clinical studies have revealed that RNase 7 and 4 are produced by α - and β -intercalated cells in renal collecting tubules and by epithelial cells of the lower urinary tract (Bender et al., 2021; Spencer et al., 2013). They are constitutively secreted into urine in physiological situations, but their expression is increased under the conditions of infection (Spencer et al., 2013). Moreover, women with a history of urinary tract infection usually have suppressed levels of urinary RNase 7 or 4 (Bender et al., 2021; Eichler et al., 2019), suggesting their importance in resisting bacterial infection. Notably, the expression of RNase 7 and 4 in the urinary tract is stimulated by bacterial factors depending on Toll-like receptor (TLR) signaling pathways as well as by the insulin-mediated PI3K/AKT signaling pathway, which partially answers the question of why people with diabetes are prone to urinary tract or kidney infections (Eichler et al., 2016). As indicated above, both proteins display a wide range of antimicrobial activities. Thus, the neutralization of RNase 7 or 4 in human urine by antibodies increases UPEC survival; when RNase 7 or 4 is silenced using siRNA, the urothelial cells are more susceptible to being infected by UPEC. These findings suggest that RNase 7 and 4 have the potential as UTI prognostic markers or as therapeutic targets for protection against bacterial infection.

In contrast to RNase 7 and 4, Becknell et al. found that RNase 6 and 3 are immune cell-derived proteins in the urinary tract (Becknell et al., 2015; Saxena et al., 2018). Although they are not routinely detected in the non-infected urinary tract, their production is significantly increased upon bacterial infection, accompanied by the massive infiltration of monocytes/macrophages and neutrophils (Becknell et al., 2015). *In vitro* antibacterial tests also suggest that RNase 6 exhibits potent antimicrobial activity against uropathogenic bacteria similar to RNase 7 (Becknell et al., 2015, 2019). Furthermore, RNase 3 is reported to have activity against the parasite, such as *Schistosoma haematobium*. Patients with bladder *Schistosoma* infection have significantly increased concentrations of urinary RNase 3, the magnitude of urinary RNase 3 is also proportional to the intensity of infection (Becknell et al., 2019). Therefore, it is believed that RNase 6 and 3 are additional weapons to control bacterial and parasitic infection in the urinary tract.

Intestinal infection

Intestinal infection is usually caused by bacteria, viruses, and parasites. Besides suppressing bacteria and viruses, RNase A family members also showed the ability to inhibit parasites in the intestine. Recent studies have revealed that RNase 3 level is significantly increased in patients infected by *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*, or *Echinococcus* (Amoani et al., 2019; Hotz et al., 2022). Combined the data of RNase 3 in urinary tract infection, RNase 3 may be developed as a general biomarker for parasitic infection. The parasitocidal activity of RNase 3 depends on both its cationic nature and enzymatic activity, and its median lethal concentration (LC₅₀) to *Leishmania* is 3 μ M (Attery and Batra, 2017). In addition, in mouse *Trichuris muris* infection model, which is used to study human gastrointestinal nematode infection, D'Elia et al. found mouse Ang 4, an intestinal-specific homologous protein of human RNase 5, was significantly increased (D'Elia et al., 2009). The expression of Ang 4 is also up-regulated after

the challenge of *Salmonella*, a Gram-positive intestinal pathogen bacterium, and *in vitro* study showed that 28 μM mouse Ang 4 could strongly affect bacterial membrane integrity and kill them (Walker et al., 2013). However, other family members in intestinal infection need to be further investigated.

INFLAMMATORY DISEASES

In addition to antimicrobial activity, RNases exert immunomodulatory activities, such as chemo-attractants, damage-associated molecular patterns (DAMPs or alarmins), and immune cell activators. Also, they participate in the clearance of extracellular RNA. Indeed, increasing evidence indicates that RNases may play an important role in inflammatory diseases and may serve as potential biomarkers or therapeutic targets for these diseases. Here, we summarize the current knowledge about the immunomodulatory activities of RNases and highlight their potential roles in clinical translation.

Asthma

Asthma is a heterogeneous and complicated respiratory disease that is usually characterized by chronic inflammation of the airway. Type 2 asthma is characterized by eosinophilic inflammation wherein eosinophil-derived granule proteins, including RNase 2 and 3, play a significant role in the immune responses (Lu et al., 2018; Rosenberg, 2015). Eosinophil-derived granule proteins are suggested to be better indicators of eosinophil activation status as opposed to absolute eosinophil numbers (Kim et al., 2011). Kim et al. reported that serum RNase 2 and 3 levels are significantly higher in childhood atopic asthma than in non-atopic asthma or in the healthy control group (Kim et al., 2010). Similar results were reported in adult asthmatics (Lee et al., 2019). A more detailed study conducted by Granger et al. reported that these two proteins are associated with different asthma symptoms. Specifically, high RNase 2 levels are associated with wheezing and breathlessness and can predict persistent asthma, whereas high RNase 3 levels tend to correlate with high neutrophil counts and chronic bronchitis (Granger et al., 2022). Interestingly, in patients with severe eosinophilic asthma, RNase 2 levels are positively associated with clinical treatment responses to the anti-interleukin-5 monoclonal antibody mepolizumab (Howarth et al., 2020). According to these observations, RNase 2 and 3 could be developed as clinical markers to determine suitable personalized therapeutic management. Although RNase 2 and 3 levels are closely associated with asthma, their functions in asthma have not been fully elucidated. Up to now, it is thought that the release of eosinophil granule-derived cytotoxic proteins is critical for inflammation-associated tissue damage in asthma (Saito et al., 2014). However, we hold the opinion that cytotoxicity may only be a side effect of the high levels of RNase 2 and 3 as they have strong basic protein properties, and their initial motivation should be protective of the human body, such as signaling molecules that regulate the reactivity of immune cells or as antimicrobial proteins that inhibit pathogenic microorganism.

RNase 5 is detected in induced sputum supernatant or exhaled breath condensates collected from children with asthma, and its level is increased compared to that in healthy volunteers (Grzela et al., 2016). RNase 5 is a well-known angiogenic factor that induces the activation, proliferation, and migration of endothelial cells and smooth myocytes. These findings match the pathological process of airway remodeling and neovascularization associated with asthma progression (Grzela et al., 2016). Moreover, RNase 5 in induced sputum supernatant or exhaled breath condensates can be a potential non-invasive marker of disease progression.

Inflammatory skin diseases

Atopic dermatitis (AD) and psoriasis are common chronic inflammatory diseases of the skin. Through many years of diverse population-based studies, serum RNase 2 and 3 levels were found to be significantly increased in recalcitrant AD and psoriasis, with active eosinophil infiltrates and extracellular granule protein deposits in the skin and increased blood eosinophil levels (Kim et al., 2017). At the cellular level, Amber et al. found that RNase 2 and 3 could significantly increase the expression of IL-5, eotaxin-1, C-C motif chemokine ligand 5, and metal metalloprotease 9, which are known to be involved in the pathogenesis of bullous pemphigoid (Amber et al., 2018). In addition, RNase 2 and 3 are cytotoxic to keratinocytes as they induce reactive oxygen species (ROS) formation and apoptosis through a mitochondria-dependent pathway.

In contrast, Miyagaki et al. found that serum RNase 5 was significantly decreased in patients with psoriasis but not in those with AD (Miyagaki et al., 2012). Moreover, in patients with psoriasis, RNase 5 expression in dermal vascular endothelial cells was decreased compared with that in healthy controls (Miyagaki et al.,

2012). RNase 5 is reported to effectively inhibit polymorphonuclear leukocytes (PMNL) degranulation (Tschesche et al., 1994). Thus, its deficiency in psoriatic skin might enhance PMNL degranulation in lesional skin, consequently activating downstream inflammatory reactions and promoting psoriasis progression.

RNase 7 is one of the major antimicrobial peptides present in the skin. Its expression is significantly upregulated in patients with psoriasis and AD and is even higher in patients with acute exacerbation of AD than in those with chronic AD (Harder et al., 2010). However, its antimicrobial activity is affected by the extracellular environment. Kopfnagel et al. reported that self-DNA concentration in lesional AD skin is higher than in healthy skin, which attenuates the antimicrobial activity of RNase 7 (Kopfnagel et al., 2021). Although the bactericidal action of RNase 7 is partly inhibited, it is reported to exert important functions in innate immunomodulation. In human plasmacytoid dendritic cells (pDCs), RNase 7 promotes TLR9-mediated self-DNA sensing and upregulates the production of interferon- α (IFN- α), IL-6, and tumor necrosis factor- α (TNF- α), leading to the continuous activation of antiviral immune responses and driving autoimmunity in psoriasis (Kopfnagel et al., 2018). Furthermore, RNase 7 stimulates keratinocytes to induce an antiviral defense system through a similar mechanism (Kopfnagel et al., 2020). RNase 7 has also been reported to regulate adaptive immunity. The evidence showed that RNase 7 treatment could selectively reduce the expression of TH2 cytokines (IL-13, IL-4, and IL-5) in activated human CD4⁺ T-cells and TH2 cells. However, T cells isolated from patients with AD are less sensitive to RNase 7 and secrete higher levels of IL-13, which might promote the pathogenesis of atopic dermatitis (Kopfnagel et al., 2017).

Inflammatory bowel disease

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a digestive tract disease characterized by chronic and relapsing intestinal inflammation. More than 20 years ago, Saitoh et al. found that enhanced levels of fecal RNase 3 and other eosinophil granule-derived proteins can reflect disease activity in IBD (Saitoh et al., 1999). Moreover, Loktionov et al. found that the colorectal mucus from patients with IBD contained higher levels of several antimicrobial peptides, including RNase 2 (Loktionov et al., 2017). Amcoff et al. found that fecal RNase 2 levels were increased both three months prior and at relapse in UC (Amcoff et al., 2019). This suggests that RNase 2 and 3 are potential non-invasive markers for the diagnosis or activity monitoring of IBD. However, their underlying mechanisms in IBD pathogenesis need to be studied systematically.

Recently, our work has revealed the mechanisms by which RNase 5 prevents the development of IBD. On one hand, RNase 5 is secreted into the gut lumen by Paneth cells to maintain the homeostasis of gut microbiota. It can inhibit harmful α -Proteobacteria and thus promote the growth of Lachnospiraceae (Sun et al., 2021). On the other hand, myeloid cells could secrete RNase 5 as a ligand that interacts with its acceptor plexin-B2 in epithelial cells to maintain intestinal barrier integrity (Bai et al., 2020). Notably, the RNase 5 levels in both intestinal tissues and feces are significantly reduced in patients with IBD compared to those in healthy controls, which is different from RNase 2 and 3. These differences in expression pattern changes may indicate their different roles in IBD.

Sepsis

Sepsis is characterized by the widespread activation of host innate immune responses, leading to an excessive inflammatory response. Considering RNase A family members are important components of the innate immune system, Martin et al. carried out an original study to investigate the serum RNases 1, 3, and 7 concentration in sepsis and found that these RNases are elevated in patients with sepsis (Martin et al., 2016). Functionally, RNase 1 serves as a natural blood vessel-protective antagonist of extracellular RNA (eRNA), which is released by damaged tissue in sepsis and may cause a severe pro-inflammatory reaction (Martin et al., 2016). Moreover, Zechendorf et al. confirmed that RNase 1 attenuates septic cardiomyopathy and cardiac apoptosis by specifically cleaving eRNA in a murine model (Zechendorf et al., 2020). However, the exact roles of RNases in sepsis are not clear yet, and future studies are needed to reveal their functions and working mechanisms in sepsis.

NEUROLOGICAL DISEASES

Many members of the RNase A family are involved in neurological disorders but have diverse functions. Two members, RNase 2 and 3, can cause the Gordon phenomenon (a syndrome of muscular rigidity, ataxia, and progressing to severe paralysis) when injected intraventricularly into guinea pigs or rabbits

(Rosenberg, 2008a). Subsequent studies have confirmed their high cytotoxicity to Purkinje cells, which are the sole output neurons of the cerebellar cortex playing pivotal roles in coordination, control, and learning of movements. The neurotoxicity of RNases 2 and 3 is found to be dependent on enzymatic activity and positive charge, but the detailed underlying mechanisms remain unclear (Rosenberg, 2008a). Clinical investigations have found that serum RNase 2 levels are significantly increased in amyotrophic lateral sclerosis (ALS) (Liu et al., 2013). The neurotoxic activities of eosinophil proteins and development of the Gordon phenomenon may therefore result from the combined action of cytotoxic RNase 2 and 3. However, these findings need to be verified *in vivo* using transgenic mice and disease models.

In contrast, RNase 4 and 5 exhibit neuroprotective functions by promoting neuronal survival under stress. These two genes are in the same locus and share the same promoters, followed by two distinct exons encoding RNase 5 and RNase 4, respectively. It has been reported that 0.5–1% of ALS and Parkinson's disease (PD) cases are associated with mutations in RNase 5, whereas only one SNP site (rs3748338) in RNase 4 is associated with ALS (Padhi et al., 2019; Sheng and Xu, 2016). Experimental studies have confirmed that RNase 5 can promote neurite growth and pathfinding, activate microglia, and modulate astrocyte function (Subramanian et al., 2008). Mechanistically, RNase 5 regulates the cleavage of tRNA into smaller fragments termed tiRNA, which in turn influences protein translation and subsequently promotes cell survival (Lyons et al., 2018). Although most RNase 5 mutations that are segregated with ALS do not significantly alter the secondary structure or conformational stability of RNase 5, they disrupt its ribonucleolytic activity or subcellular localization, which further affects the tiRNA production. RNase 4 was found to stimulate the formation of neurofilaments from mouse embryonic cortical neurons and to protect against hypothermia-induced degeneration. However, the mechanisms responsible for the neuroprotective activity of RNase 4 are not completely understood. Importantly, systemic treatment with RNase 4 or 5 proteins in SOD1^{G93A} transgenic mice (an ALS model) slowed the weight loss and symptom progression. Therefore, haploinsufficiency of RNase 4 and 5 might be a risk factor, and these two proteins, either alone or together, might be beneficial in treating neurodegenerative disorders.

Considering the important role of RNA processing and metabolism in neurological diseases (Thelen and Kye, 2019), RNase A family members can exert either neurotoxic or neuroprotective roles in neurological diseases based on their ribonucleolytic activity. Meanwhile, ribonuclease activity-dependent and -independent pathways do not mutually exclude each other. For example, cell surface receptors, including heparan sulfate proteoglycan syndecan-4, Plexin-B2, and epidermal growth factor receptor (EGFR), are known to be involved in the binding, internalization, and signaling of RNase 5 (Liu et al., 2018). As these signaling pathways are also known to promote neurogenesis (Junqueira Alves et al., 2021; Luo et al., 2016), it is likely that an enzymatic activity-independent pathway may be involved in the neuroprotective effect of RNase 5, and it will be interesting to explore this possibility in the future.

CANCER

Similar to their roles in neurological diseases, RNases have opposite effects on cancer, including cytotoxicity and protection. RNases, including RNase 1, artificial dimeric bovine seminal RNase, non-mammalian RNase onconase, can translocate into the cytosol and evade ribonuclease inhibitor (RNH1), and inhibit protein synthesis by cleaving rRNA, tRNA, or mRNA, leading to altered gene expression at different levels and triggering cell death (Castro et al., 2021; Gotte and Menegazzi, 2019). RNase 2 and 3 can also inhibit tumor cell growth by their cytotoxicity *in vitro* (Davis and Rothenberg, 2014). eRNAs are found to be increased around tumor tissues and have the capacity to promote tumor cell progression through proinflammatory stimuli (Fischer et al., 2013; Wang and Tang, 2021). Fischer et al. found that RNase 1 could effectively clean eRNA and destroy the tumor microenvironment, thereby inhibiting tumor cell trafficking and progression (Fischer et al., 2013). Based on the knowledge gained from the mechanisms of the natural cytotoxic RNases, many recombinant antitumor RNase variants have been generated, some of which have reached phases II/III clinical trials (Kanwar and Kumar, 2017).

In contrast, some RNases exhibit cancer-promoting activity through multiple mechanisms. The most studied oncogenic molecule is RNase 5 (generally known as angiogenin), which was first isolated from the conditioned media of HT-29 human colon adenocarcinoma as a tumor angiogenic factor (Sheng and Xu, 2016). RNase 5 can increase tumor angiogenesis, thus providing sufficient nutrients and oxygen to support tumor cell proliferation. Further exploration has revealed that RNase 5 promotes cancer cell growth, survival, and metastasis (Sheng and Xu, 2016). Mechanistically, RNase 5 could translocate into the nucleus

and accumulate in the nucleolus, thereby promoting 47S pre-rRNA transcription by binding the Angiogenin Binding Element (ABE, a 21-mer CT repeated sequence) at rDNA intergenic sequence and the upstream control element at rDNA promoter (Sheng et al., 2014; Xu et al., 2003). RNase 5 also enhances tumor cell survival under stress through RNase 5-mediated tRNA production. Li et al. found that RNase 5 promotes colorectal cancer metastases in a tRNA-dependent manner (Li et al., 2019). In 2017, Hu's team and Hung's team found that RNase 5 binds to cell membrane receptors (Plexin-B2 or EGFR), thus activating their downstream pathways and promoting tumor development (Wang et al., 2018a; Yu et al., 2017). Besides RNase 5, recent studies have indicated that RNase 1, 4, and 7 serve as ligands for receptor tyrosine kinase (RTK) ephrin A4 (EphA4), AXL receptor tyrosine kinase (AXL), C-ros oncogene 1 (ROS1) in solid cancers to promote tumor progression, respectively. Together, these findings reveal an unconventional ligand-receptor relationship and the role of the RNase A superfamily in tumor progression.

Four RNase A family members, including RNase 1, 2, 3, and 5, were identified in human serum (Barrabés et al., 2007; Granger et al., 2022; Yu et al., 2021). A correlation between serum RNase levels and cancer has been reported in patients with cancer. In a meta-analysis of 37 case-control studies, we found that serum RNase 5 levels in patients with cancer are significantly higher than those in healthy controls (Yu et al., 2018). Another meta-analysis conducted by Aalami et al. revealed that urinary RNase 5 is significantly increased in patients with bladder neoplasms, and diagnostic precision analysis indicated its potential as a biomarker for bladder cancer (Aalami et al., 2021). In fact, as early as 1976, it has been reported that serum RNase and ribonuclease activity levels are upregulated in different types of tumor, especially in pancreatic cancer (Reddi and Holland, 1976); however, as serum RNase could be derived from many organs and tissues, it was considered neither sensitive nor specific enough for this purpose (Weickmann et al., 1984). Until 2007, Barrabés et al. found a subset of pancreatic cancer-associated glycoforms of RNase 1, which could be an effective biomarker for pancreatic cancer (Barrabés et al., 2007). These results indicate that certain RNases need further evaluation to determine whether they can serve as a new class of diagnostic biomarkers for tumors.

CARDIOVASCULAR DISEASE

Emerging evidence indicates that there is a high level of RNase 1 in blood circulation, with considerable serum concentrations, reaching 0.5 $\mu\text{g}/\text{mL}$ (Garnett et al., 2019). In recent years, RNase 1 has been considered a key player in the regulation of vascular homeostasis. One of the major functions of RNase 1 is to digest eRNA (Bedenbender and Schmeck, 2020; Tosar et al., 2021), which is significantly increased upon vascular inflammation or injury. It is now clear that eRNA can act as a potent inducer of the immune response in the endothelium, resulting in increased secretion of pro-inflammatory cytokines, especially TNF- α and IL-1 β , and can greatly repress RNase 1 expression (Bedenbender et al., 2019; Bedenbender and Schmeck, 2020). The output of this feedback loop is redundant eRNA accumulation in the extracellular space and inflammation-associated vascular damage. Therefore, the RNase 1-eRNA balance is believed to play a significant role in the pathogenesis of atherosclerosis and thrombosis (Zernecke and Preissner, 2016). Supplementation with RNase 1 demonstrated a protective function in the cardiovascular system. Cabrera-Fuentes et al. used an *in vivo* mouse model and a rat heart model to find that under ischaemia/reperfusion (I/R) conditions, the damaged cardiac tissue released RNA and tumor-necrosis-factor, which contributed to I/R injury, whereas the administration of RNase 1 could significantly decrease myocardial infarction (Cabrera-Fuentes et al., 2014). In an experimental heart transplantation study, Kleinert et al. found that RNase 1 administration counteracts I/R injury and graft rejection, thus significantly prolonging rat survival after heart transplantation (Kleinert et al., 2016). Moreover, clinical investigations have shown that if patients undergo a remote ischemic preconditioning (RIPC) protocol before cardiac surgery, their levels of plasma endogenous vascular RNase 1 were significantly increased, while the concentrations of circulating eRNA and TNF- α were decreased (Cabrera-Fuentes et al., 2015). RNase 1 has thus been demonstrated to be a promising target for the development of novel treatment strategies for cardiovascular diseases.

RNase 4 has strong enzymatic activity and exhibits substrate preferences similar to those of RNase 1 (Sorrentino, 2010). Our study showed that serum RNase 4 concentrations range from 0.1 to 0.2 $\mu\text{g}/\text{mL}$ in healthy individuals (unpublished data), and might contribute considerably to partial eRNA degradation. Evidence from RNase 1 knockout mice showed that RNase 4 levels are increased, which is considered a compensation mechanism of the body to deal with eRNA accumulation (Garnett et al., 2019). Moreover, RNase 4 could induce blood vessel formation and promote angiogenesis. It is reported that the RNase 4 K40A variant, which decreases the catalytic ribonucleolytic activity by 20-fold, did not inhibit the angiogenic

activity of RNase 4; while diethylpyrocarbonate (DEPC) treatment completely abolished both ribonucleolytic and angiogenic activity of RNase 4. These results suggest that RNase 4 does not require a full enzymatic activity to induce angiogenesis but a certain degree of the enzymatic activity is essential (Li et al., 2013). Overall, our understanding of RNase 4 function in cardiovascular disease is very limited, and further studies are needed.

RNase 5 has very weak ribonucleolytic activity, which is only 1×10^{-5} compared to that of RNase1 *in vitro* (Sheng and Xu, 2016). Although serum RNase 5 concentrations are approximately 0.3 $\mu\text{g}/\text{mL}$ in healthy populations, RNase 5 might not be the main RNase maintaining eRNA homeostasis in blood vessels. However, it promotes endothelial cell proliferation and vesicle tube formation, in which weak enzymatic activity is indispensable (Sheng and Xu, 2016). Kręcki et al. found that in patients with coronary artery disease (CAD), surgical treatment (coronary artery bypass grafting) resulted in significantly decreased serum RNase 5 levels, but had no effect in the medically treated group (Kręcki et al., 2010). Recently, RNase 5 was reported to be significantly increased in the plasma of patients with acute coronary syndrome (ACS); high RNase 5 levels were also predictive of adverse events (Tello-Montoliu et al., 2007). However, Höbaus et al. found that in patients with peripheral artery disease, the serum concentration of RNase 5 was not associated with outcome prediction (Höbaus et al., 2021). Next, more mechanistic studies are needed to support the clinical relevance between RNase 5 and cardiovascular disease.

Taken together, most findings support the probability that RNases are involved in cardiovascular diseases as a protective factor. In cardiovascular disease, redundant eRNA could induce excessive inflammatory response. The levels of serum RNase 1, 4, and 5 were determined at a concentration 0.5, 0.2, and 0.3 $\mu\text{g}/\text{mL}$, respectively, which belong to a group of serum proteins with medium-to-high abundance (medium abundance: 0.1–1 $\mu\text{g}/\text{mL}$; high abundance: >1 $\mu\text{g}/\text{mL}$) (Ignjatovic et al., 2019). The relatively high concentration of serum RNases may predominantly contribute to minimizing eRNA level in the circulatory system. Thus, targeting the RNases-eRNA balancing may be a promising therapeutic strategy against cardiovascular diseases.

CONCLUSIONS AND PERSPECTIVES

The human RNase A family has diverse functions, and their expression disorders or loss-of-function mutations are associated with the diseases mentioned above. Encouragingly, studies have revealed some important molecular mechanisms of RNases action in diseases. It not only reveals the pathogenesis of diseases but also provides a potential diagnosis marker or treatment target for the diseases (Table 2).

The role of the RNase A family in human health and disease is still not completely revealed. Up to now, the basic and clinical research evidence of RNase in disease were still not comprehensive and systematic. Especially, the knowledge for RNase 4, 6, and 8 was relative lack. Therefore, subsequent studies could focus more on these members, including the non-canonical RNase 9–13, under specific physiologic and pathologic conditions. In addition, although an altered RNases expression pattern in disease was observed, more evidence is required to distinguish if this is a cause or a consequence of disease. In-depth mechanism studies, such as combined transgenic animals with certain disease models, will be helpful to solve this question. On the other hand, we need to consider the role of RNases in specific tissues or organs with multiple diseases. For example, chronic inflammation associated with certain infectious diseases contributes significantly to carcinogenesis. RNases are functionally linked to infection, inflammation, and cancer in a specific organ, therefore, it is necessary to study the role of RNase throughout the whole disease process.

Notably, RNases could show completely opposite biological functions in physiological and pathological conditions. For example, RNase 5 exerted different roles in neurological disease (beneficial) and in cancer (harmful). RNase 2 exhibited tumor-cell-killing effect (beneficial) but contributed to inflammation-associated tissue damage in asthma (harmful). RNase 1 used to be identified as a tumor suppressor (beneficial) by degrading eRNA in the tumor microenvironment; however, it is reported that RNase 1 can promote breast cancer progression and stemness (harmful) by binding and activating the EphA4. Although the exact mechanisms of these opposite activities remain to be elucidated, researchers are beginning to realize that the targeted cell types, subcellular localization, interaction partners, and expression level are responsible for this phenomenon. As RNase 5 is the most investigated RNase member, it is clear that the protective role of RNase 5 in neurons is mediated by producing a class of bioactive tiRNAs that restricts protein synthesis in the cytoplasm, while the tumor cell growth-promoting activities of RNase 5 are mediated by promoting 47S

Table 2. Summary of functions, mechanisms, and applications of RNases in diseases

Disease	RNase	Expression during disease	Function	Mechanism of action	Potential applications	Reference
Respiratory tract infection	RNase 2, 3	Upregulated	Inhibit RSV	Ribonuclease activity and other unique ability needed	Biomarker; treatment reagent	(Domachowski et al., 1998a; Domachowski et al., 1998b)
	RNase 5	Unknown	Promote RSV proliferation	By producing 5'tiRNA-Glu ^{CTC} to inhibit the expression of APOER2	Therapeutic targets	(Wang et al., 2013)
	RNase 3, 6, 7	Upregulated	Anti-bacteria	Inhibit bacteria growth extracellularly; Induce autophagy process in macrophages	Treatment reagent	(Amatngalim et al., 2015; Li et al., 2019)
Skin infection	RNase 1, 4, and 7	Upregulated	Anti-bacteria	Positive charge dependent	Treatment reagent	(Abtin et al., 2009; Harder and Schroder, 2002)
	RNase 5	Unknown	Anti-fungi	Ribonuclease dependent	Treatment reagent	(Abtin et al., 2009)
Intestinal infection	RNase 3	Upregulated	Anti- parasite	Depend on both its cationic nature and enzymatic activity	Treatment reagent	(Amoani et al., 2019)
	Mouse Ang 4 (homolog of RNase 5)	Upregulated	Anti-bacteria	Unknown	Biomarker; treatment reagent	(Walker et al., 2013)
Urinary tract infections	RNase 4, 6, and 7	Upregulated	Anti-bacteria	Positive charge dependent	Biomarker; treatment reagent	(Becknell et al., 2015; Bender et al., 2021; Spencer et al., 2013)
	RNase 3	Upregulated	Anti-parasite	Unknown	Biomarker	(Saxena et al., 2018)
Asthma	RNase 2, 3	Upregulated	Participate in immune responses	Cause tissue damage at the site of inflammation	Biomarker	(Kim et al., 2013)
	RNase 5	Upregulated	Airway remodeling and neo-vascularization	Promote endothelial cells and smooth myocytes proliferation and migration	Biomarker; treatment reagent	(Grzela et al., 2016)
Atopic dermatitis, psoriasis and bullous pemphigoid	RNase 2, 3	Upregulated	Participate in immune responses	Enhance cytokines expression, induce ROS and apoptosis	Biomarker	(Amber et al., 2018; Kim et al., 2017)
	RNase 5	Downregulated in patients with psoriasis	Promote psoriasis progression	Inhibit polymorphonuclear leukocytes degranulation	Biomarker; treatment reagent	(Miyagaki et al., 2012)
	RNase 7	Upregulated	Modulate innate immune	Promote TLR9-mediated self-DNA sensing	Biomarker; treatment reagent	(Kopfnagel et al., 2017; Kopfnagel et al., 2018)
Inflammatory Bowel Disease	RNase 2, 3	Upregulated	Unknown	Unknown	Biomarker	(Liu et al., 2013; Saitoh et al., 1999)
	RNase 5	Downregulated	Inhibit IBD	Maintain gut microbiota, enhance intestinal barrier integrity	Biomarker; treatment reagent	(Bai et al., 2020; Sun et al., 2021)

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Table 2. Continued

Disease	RNase	Expression during disease	Function	Mechanism of action	Potential applications	Reference
Sepsis	RNase 1, 3, 7	Upregulated	Attenuate septic cardiomyopathy and cardiac apoptosis	Digest eRNA	Treatment reagent	(Martin et al., 2016; Zechendorf et al., 2020)
Neurological diseases	RNase 2 and 3	Upregulated	Neurotoxicity	Enzymatic activity and the positive charge dependent	Biomarker	(Liu et al., 2013; Rosenberg, 2008a)
	RNase 4 and 5	unknown	Protect neurons	RNase 5 helps motor neuron survival by cutting tRNA into tiRNA	Biomarker; treatment reagent	(Li et al., 2013)
Cancer	RNase 1	Upregulated	Inhibit cancer; promote breast cancer initiation	Kill cancer cells, digest eRNA; bind with EphA4	Biomarker; treatment reagent	(Fischer et al., 2013; Gotte and Menegazzi, 2019)
	RNase 5	Upregulated	Promote cancer growth, survive, and migration	Promote 47S pre-rRNA transcription, product functional tiRNA, bind with PlenxinB2 and EGFR	Biomarker; treatment reagent	(Wang et al., 2018b; Yu et al., 2017)
Cardiovascular disease	RNase 1	Downregulated	Protect cardiovascular system	Digest eRNA	Treatment reagent	(Cabrera-Fuentes et al., 2015; Garnett et al., 2019; Tosar et al., 2021)
	RNase 5	Upregulated	Protect vascular endothelium	Promote endothelial cell proliferation and vesicle tube formation	Biomarker	(Krećki et al., 2010; Tello-Montoliu et al., 2007)

ribosomal RNA (rRNA) transcription through epigenetic activation of the ribosomal DNA promoter in the nucleus. Moreover, ribonuclease inhibitor (RNH1) controls both the localization and the activity of RNase 5 (Pizzo et al., 2013). Thus, the different subcellular localization and RNA processing of RNase 5 lead to distinct biological functions. It will be worthwhile to determine the mechanisms of other members with opposite activities.

Importantly, many of their functions have great application value; however, clinical translational studies are lagging. For example, their host defense function can be exploited to maintain the balance of microbiota and to treat infection disease, as an alternative to antibiotics. Their tumor-cell-killing ability can be used to inhibit tumor growth with the premise of improving specificity and efficiency; or to attenuate tumor development, by specifically blocking RNases associated tumor-promoter signaling pathways; it is also expected to improve the outcomes of patients with cardiovascular disease by take advantage of RNase's cardiovascular-protection ability.

All in all, there is a strong belief that further understanding of human RNase A family will be the prerequisite for improving and expanding the potential therapeutic and diagnostic options.

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed, Google Scholar, and references from relevant articles using the search terms "RNase A family," "RNase 1," "RNase 2 or EDN," "RNase 3 or ECP," "RNase 4," "RNase 5 or ANG," "RNase 6," "RNase 7," and "RNase 8." They were selected based on their relevance to the topic. Only articles published in English between 1976 and 2022 were included.

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AUTHOR CONTRIBUTIONS

JS and DS contributed to the conception and design of the review. DS and CH performed the literature search and analysis. DS, CH, and JS drafted and critically revised the article. All authors contributed to the approved and submitted version.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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