Editorial

Vertebrate RNase 1 homologs: potential regulators of extracellular RNAs

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Recently, much data has been accumulated on extracellular RNAs (exRNAs) circulating in the blood plasma of mammals, and on alteration of their concentration/patterns occurring in pathological states including inflammation, trauma and oncological diseases (1,2). The spectrum of RNA detected in blood plasma is quite wide: fragments of mitochondrial RNA, rRNA, tRNA, mRNA and small non-coding RNA (snoRNA, miRNA) (3). Biological activity of exRNAs can be summarized as follows: exRNA can enter the nucleus of the host cell *in vitro* and *in vivo*, and can be transcribed and translated into proteins; small non-coding RNA, in particular miRNA, can regulate the processes of control tissue development and malignant transformation at the organism level by paracrine and endocrine regulation (4,5). Given the variety of mechanisms of biological activity of RNAs, it can be confidently asserted that exRNAs are important participants in cell-to-cell communication, and switchers of intracellular cascades that control cell proliferation and differentiation. This indicates involvement of exRNAs in the control of many processes, including immune response to pathogens and carcinogenesis.

In connection with elucidating such an important role of exRNAs, a new wave of interest in enzymes capable of degrading RNAs has flared up. Endogenous ribonucleases (RNases) perform many different functions, from regulation of miRNA biogenesis to RNA decay, and their main function is maintenance of RNA homeostasis at cell level. Secreted RNases are considered to be digestive enzymes participating in gut fermentation and degradation of high molecular weight RNAs from food. RNase A, belonging to the pancreatic-type RNases (ptRNases), is an example of a digestive enzyme. RNase activity in the blood is maintained by circulating RNases, in particular, in humans and mice by RNase 1 (6), a ptRNase. Data has begun to accumulate on changes in RNase activity in blood plasma under pathological conditions, in line with the level of circulating exRNAs, suggesting an important role of circulating RNases in maintenance of RNA homeostasis in organisms. Recently, a number of proteins with RNase activity have been discovered, the exact role of which in the cell has not yet been established (7). Thus, the true physiological role of RNases is still to be unveiled.

The recent article by Raines and colleagues reported the broadest study of the function of RNase 1 across a vertebrate evolutionary spectrum, including mammalian (human, horse, cow, mouse, squirrel, cat, bat) and non-mammalian (chicken, lizard, frog) homologs (8). It was found that RNase 1 homologs exhibit pronounced differences in catalysis, pH optima for catalysis and binding efficacy with cell-surface glycans. The highest catalytic activity, but at the lowest pH optimum (6.1), was displayed by cow RNase 1 (pancreatic RNase A). Human RNase 1 was at the opposite end of the spectrum, with ~5-fold lower catalytic activity and a much higher pH optimum (7.3). Other mammalian homologs demonstrated a range of significantly lower enzymatic activities at increasingly higher pH optima. Non-mammalian homologs (chicken, anole lizard and frog) all demonstrated very low catalytic activity at relatively low pH.

RNase 1 homologs were able to degrade ssRNA;

however, enzymes from human, bat, squirrel and horse were found to display a pronounced ability to degrade dsRNA. Catalytic efficacies of human and bat RNase 1 with respect to dsRNA were ~1800-fold and ~1400-fold higher, respectively, than that of cow RNase 1. Human RNase 1 demonstrated significantly higher activity than all other homologs, except for bat RNase 1 (8).

Study of cellular uptake of fluorophore-labeled RNase 1 homologs by a nonadherent human cell-line (K-562) revealed that all of the proteins, both mammalian and non-mammalian, were taken up readily by cells (8). Human and anole RNase 1 homologs demonstrated the highest level of cellular internalization, followed by bat and squirrel RNase 1, whereas cow and frog RNase 1 demonstrated the lowest level. Efficacy in the cellular uptake of mammalian RNase 1 homologs correlated with their affinity for common cell-surface glycosaminoglycans [heparin sulfate (HS) and two forms of chondroitin sulfate]: human and bat RNase 1 showed extremely tight affinity for each glycan, while non-mammalian homologs had weak affinity.

Using ^{1}H-^{15}N-HSQC NMR spectroscopy, residues in human RNase 1 that interact with HS and phosphatidylserine (PS) were identified. The main interactions between the enzyme and both HS and PS were discovered predominantly in a polar serine-rich loop, Ser18–Thr24, as well as at residues Cys72, Asn76 and His80 of RNase 1. By comparison with mammalian RNase 1 orthologs, evolutionary changes were found among residues on the protein surface, whereas many residues participating in protein structure support, enzymatic activity and binding to RNase inhibitor were highly conserved across species. A strong correlation was found between residues predicted to be evolving faster and those residues associated with dsRNA degradation and cell-surface binding. Given the ability of RNase 1 homologs to degrade dsRNA, to enter endosomes readily and to exhibit high affinity for cell-surface glycans, and the fact that RNase 1 is the only known RNase in bodily fluids with high, nonspecific ribonucleolytic activity, RNase 1 homologs might be considered as natural regulators of exRNA level and composition (8).

In cells, various endogenous RNases participate in the control of RNA homeostasis by rejecting aberrant RNAs, controlling RNA decay and miRNA biogenesis. In this regard, a change in the level of expression or functioning of endogenous nucleases leads to the accumulation of aberrant RNAs that are incapable of performing their functions, or transcripts encoding oncogenic proteins that change the normal functioning of intracellular signaling pathways to pathological ones, leading to oncogenesis and tumor progression.

Angiogenin was the first member of the RNase A superfamily to be described as a proto-oncogene, and an increase in its level is noted in many types of cancer cell (7). There are a number of RNases such as CCR4b, XRN1 and PARN which are involved in the process of conventional RNA decay (7). They initiate mRNA degradation, and act at the stage determining mRNA turnover rate and the level of gene expression. PARN has deadenylase activity and, coupled with the destabilizing actions of RNA binding protein (RBP), can potentially act as a tumor suppressor, causing degradation of mRNAs encoding growth factors such as IL-8 and VEGF. It has been shown that defective RBP that cannot recruit PARN plays a role in malignant glioma cells (9). Also, PARN and the exosome complex are shown to be recruited by KSRP and/or DHAU, and account for the destabilization of various mRNAs, including c-jun and uPA, whose elevation is implicated in cancers (10,11). The 5'-3' exonuclease XRN1, besides deadenylation, can also initiate mRNA decay once its target transcript is decapped. XRN1 is an example of an enzyme that is implicated in cancer as a dysregulated tumor suppressor. In primary samples of osteogenic sarcoma and its derived cell lines, there is a reduction and/or depletion of XRN1 mRNA (12).

A number of endogenous RNases do not normally function in cells, but are activated by specific stress signals (RNase L, IRE1) (13,14). RNase L is involved in antiviral defense, but also in tumor suppression; mutations in this enzyme have been found in many types of tumor. Amino acid substitutions and mutations in RNase L lead to aggressive growth of metastases, and an increased risk of developing prostate cancer, colorectal cancer and pancreatic cancer (7). The tumor suppressive function of RNase L has been well characterized, especially under interferon signaling. RNase L is suggested to degrade mitochondrial mRNA, which can lead to cytochrome-c release and caspase-3 activation during apoptosis induced by interferon-α or an anti-cancer agent (15).

Recently, for RNase IRE1, in addition to its role in the control of ER stress, effects associated with oncogenesis and apoptosis have been found. IRE1 acts upstream to activate the unfolded protein response—a homeostatic signaling pathway which adjusts protein folding capacity in the ER to cellular need. So, IRE1 is an important RNase that directs cancer progression as well as cell death (16).

Growing evidence suggests an association between

dysregulation of miRNA expression and cancer. Subsequently, three major RNases involved in miRNA biogenesis (Drosha, Dicer and Ago) can have crucial effects in controlling miRNA and mRNA expression. Increased levels of Drosha and Dicer, and alteration of their intracellular localization, as well as their aberrant functioning observed in many types of cancer cell, correlate with increased levels of oncogenic miRNAs (7). Recent reports highlight altered levels of Ago2 in the deregulation of miRNA activities in cancer. Possible explanations include overexpression of Ago2 relieving the competition implemented between different miRNA species, to regulate gene expression and permit oncogenic miRNAs to better exert their effects (17). Thus, today it is clear that various RNases functioning in the cell and beyond are important for an orchestra maintaining RNA homeostasis.

This is the main reason that a broad front is underway across the world to study the antitumor potential of exogenous RNases. To date, the high antitumor potential of natural RNases of different origin has been confirmed. The most known RNases with pronounced antitumor activity are: BS-RNase from bull testes (18), the amphibian RNase onconase from oocytes of Rana pipiens (19) and modified variants of RNase 1 from humans (20) belonging to the RNase A superfamily, and the microbial RNases barnase from *Bacillus amyloliquefaciens* (21) and barnase from *Bacillus pumilus* (22) belonging to the RNase T1 superfamily. There are variants of RNase 1 that can act as chemotherapeutic agents against cancer cells (23), including prototypes now in clinical trials (24).

A lot of data has been accumulated which confirms that targets for exogenous RNases can be tumor-associated RNAs both in the tumor cell and exRNAs (25). This suggests that exogenous RNases can refill the level and/or function of endogenous RNases interrupted in the tumor cell, and participate in the control of tumor-associated RNAs. The discovery of the role of exRNAs in tumor dissemination has suggested that they could also be targets for exogenous RNases. Exogenous RNases, by reducing the concentration of tumor-associated exRNAs in the bloodstream, can reduce the metastatic burden and, as a consequence, the rate of dissemination of the tumor. From the data of Raines and colleagues (8), we can speculate that RNase 1 in humans and other mammals plays a broad regulatory role via the degradation of exRNAs, and thus can participate in RNA homeostasis in the organism, controlling critical processes such as hemostasis, inflammation and tumor progression.

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Footnote

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