ACS central science

FIRST REACTIONS

A Sweet Delivery for a Really Bitter Pill: Globo H and RNase 1

Masahiro Hosono

Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Sendai, Japan

Masahiro Hosono discusses the work from Eller et al. explaining the mechanism of RNase 1 cytotoxicity and implications for cancer treatment.

Herodotus, the ancient Greek historian, called Egypt the "Gift of the Nile" for the myriad ways that the river supported the growth and development of a flourishing Egyptian culture. Today, biochemists can say ribonuclease A (RNase A) is the "Gift from Anfinsen". The study of this classic enzyme has produced four Nobel laureates since 1972, starting with Christian Anfinsen himself. The RNase A superfamily (also termed the vertebrate-secreted RNase superfamily by D'Alessio) is the largest group of ribonucleolytic enzymes sharing a characteristic peptide motif, CKXXNTF, as well as an H-K-H catalytic triad in the active site.

Several enzymes in this family possess antitumor activity, for which they are being investigated for chemotherapy as well as for their distinct mechanism compared to current DNA-targeted anticancer drugs. The mechanism of RNase-derived cytotoxicity progresses as follows: first, highly cationic RNase molecules associate with anionic membrane components, leading to their internalization in the cytosol. The RNases then degrade cellular RNA while evading being trapped by the intrinsic RNase inhibitor (RI). The result is the induction of apoptosis in the relevant cells.[1] In this issue, Raines and collaborators report Globo H hexasaccharide as a newly identified ligand of cytotoxic RNase.[2] This finding represents a first in the identification of specific cell surface ligand/receptor pairs for RNase and presents a potential mechanism for its membrane transport.

The authors started by performing a comprehensive screening with glycan array. In this method, they used RNase A and its human homologue RNase 1 (human pancreatic RNase), and picked up two oligosaccharides, Globo H and SSEA-4, as tentative ligand candidates among 264 mammalian cell surface glycans (Figure 1). Globo H (also known as CaMBr1 or GL6, and structurally the same as fucosyl Gb5) was first reported by Hakomori's group in cultured human teratocarcinoma cells in 1983.[3] This hexasaccharide is the cancer antigen overexpressed on various epithelial cancer cells including breast, colon, prostate, and lung. It is currently regarded as a promising target for cancer vaccines. SSEA-4 is a stage-specific embryonic antigen, structurally the same as sialyl Gb5. Interestingly, Globo H was the only fucosylated glycan that showed a significant affinity for RNase A.

Subsequently, they did kinetic analyses and determined that Globo H had higher affinity for RNase 1 than RNase A, despite an 82% sequence homology. From there, the authors needed to show the biological relevance of the affinity. They demonstrated that Globo H was specifically involved in both recognition and internalization of the RNase molecules resulting in the known cytotoxicity. To generate a Globo H deficient breast cancer cell, small molecule fucosyltransferase inhibitors were applied. When exposed to RNase 1, these cells were more resilient than unmodified ones and survived at a higher rate. To provide further evidence via a competition assay, the authors then used an antibody specific against Globo H to block it from other antigens, and again improved RNase1 survival was observed.

Interestingly, Globo H was the only fucosylated glycan that showed a significant affinity for RNase A.

Finally, the authors identified portions of the protein that contribute to the interaction between the RNase molecule and sugar chain, using $^{1}H,^{15}N$-HSQC NMR spectrometry analysis. The binding sites of RNase 1 to Globo H are far from its catalytic center. When the pH was lowered, such as in the endosome, the putative binding pocket was narrowed, and the affinity for the ligand weakened. Taken together, these data provide a coherent story of how RNase interacts with the cells and releases to the cytosol.

Published: July 13, 2015

DOI: 10.1021/acscentsci.5b00249
ACS Cent. Sci. 2015, 1, 166−167

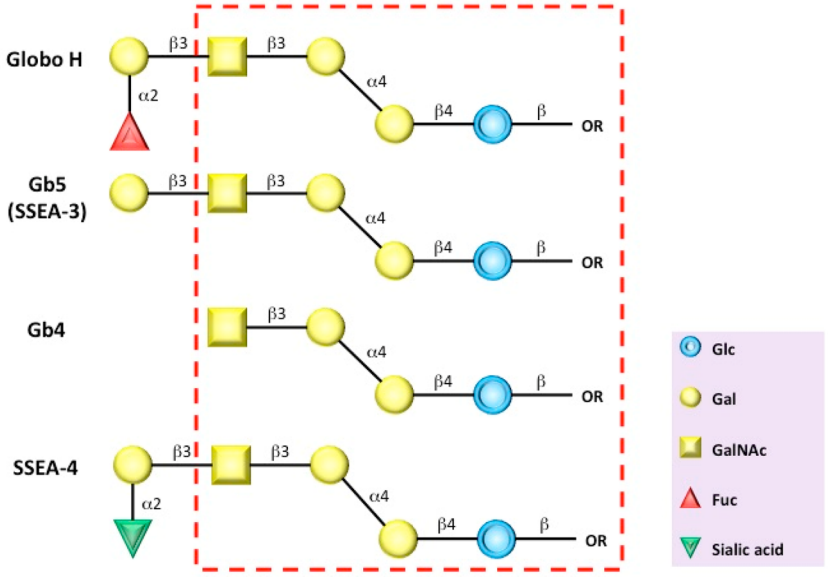

Figure 1. Structure of Globo-series saccharides.

However, some unsolved problems still remain. Under what mechanism is RNase internalized? Where on the cell surface does the Globo H bearing membrane molecule reside? An endocytosis receptor? Is the carrier of Globo H protein or lipid? This work, and answers to the questions above, will help researchers drive Globo H toward clinical use. It will be necessary to identify efficient means to direct the enzyme's molecules to the target cells and to improve their inherent internalization capabilities. One can imagine the antibody−enzyme conjugates as well as cell penetrating peptides to address the respective issues. Since Globo H is already an established cancer marker, it would give cytotoxic RNases a therapeutic advantage through innate selectivity, making this strategy targeting RNA extremely exciting. This new approach differs markedly from known genotoxic chemotherapies and could reduce serious side effects and minimize genetic damage.

Since Globo H is already an established cancer marker, it would give cytotoxic RNases a therapeutic advantage through innate selectivity, making this strategy targeting RNA extremely exciting.

On a final, more philosophical note: Onconase (ONC) from *Rana pipiens* is another member of the RNase A superfamily.[4] It, too, presents severe cytotoxicity in cancer cells, including malignant mesothelioma, due to its very low affinity with RI. Leczyme, from *Rana catesbeiana,* by contrast, shows similar cytotoxicity as ONC, but was instead originally found as sialic acid-binding lectin possessing cell agglutination activity.[5] The current finding that RNase A shows higher affinity to a neutral fucosylated glycan than an acidic sialylated one is, then, very interesting. It raises the possibility that RNase could simply be considered another "leczyme" from its method of recognizing carbohydrates (Figure 2). Although there is no doubt that the new findings of RNase ligand Globo H are really epoch-making, how to untangle these classifications and apply these newfound interactions to an RNase-mediated therapy will need to be addressed in the future.

Figure 2. 3-D structures of four RNases.

Author Information
E-mail: mhosono@tohoku-pharm.ac.jp.

REFERENCES

(1) Ribó, M.; Benito, A.; Vilanova, M. *Nucleic Acids Mol. Biol.* **2011**, *26*, 55.
(2) Eller, C. H.; Chao, T.-Y.; Singarapu, K. K.; Ouerfelli, O.; Yang, G.; Markley, J. L.; Danishefsky, S. J.; Raines, R. T. Human Cancer Antigen Globo H Is a Cell-Surface Ligand for Human Ribonuclease 1. *ACS Cent. Sci.* **2015**, DOI: 10.1021/acscentsci.5b00164.
(3) Kannagi, R.; Levery, S. B.; Ishigami, F.; Hakomori, S.; Shevinsky, L. H.; Knowles, B. B.; Solter, D. *J. Biol. Chem.* **1983**, *258*, 8934.
(4) Ardelt, W.; Mikulski, S. M.; Shogen, K. *J. Biol. Chem.* **1991**, *266*, 245.
(5) Tatsuta, T.; Sugawara, S.; Takahashi, K.; Ogawa, Y.; Hosono, M.; Nitta, K. *BioMed Res. Int.* **2014**, DOI: 10.1155/2014/421415.

DOI: 10.1021/acscentsci.5b00249
ACS Cent. Sci. 2015, 1, 166−167