Hepatocyte specific RNase H1 knockout mice: Clarifying functions of mammalian RNase H1

Stanley T. Crooke*, Wen Shen and Xue-hai Liang

Stanley T. Crooke, Ionis Pharmaceuticals Inc., 2855 Gazelle Court, Carlsbad, CA 92010, USA

ABSTRACT

Human RNase H1 cleaves RNA only when the RNA is present in a DNA-RNA heteroduplex. Previous efforts to create RNase H1 knockout mice resulted in embryonic lethality, but demonstrated that RNase H1 is required for mitochondrial function. We constructed viable constitutive hepatocyte liver specific RNase H1 knock out mice by coupling the Cre recombinase to an albumin promoter as albumin is not expressed till late in gestation. We also constructed hepatocyte specific tamoxifen inducible hepatocyte knockout mice.

Our studies demonstrate that mammalian RNase H1 is required for transcription of mitochondrial ribosomal DNA and removal of R Loops. The absence of RNase H1 leads to mitochondrial dysfunction and hepatocyte apoptosis and liver dysfunction. Subsequently, a clone of hepatocytes that lost the Cre recombinase and thereby regained RNase H1 expression emerged, supporting the analysis of events leading to liver regeneration. RNase H1 is confirmed to be responsible for the pharmacological effects of DNA-like antisense drugs as well.

Text

Human cells have two enzymes that specifically cleave RNA only when it is in a hetero-duplex with DNA, RNase H1 and H2. The specificity for cleavage of the RNA present in a duplex with DNA is effected by sensing the duplex geometry (DNA-RNA duplexes differ structurally from DNA-DNA or RNA-RNA duplexes), by sampling the minor groove of the duplexes to assure that the dimensions are those of a DNA-RNA duplex and by using the 2’ OH present in RNA as a key component of the catalytic mechanism. RNaseH2 is quite abundant, accounting for most of the RNase H activity in mammalian cells is localized to the nucleus and, in fact, is tightly bound to chromatin. In contrast, RNase H1 is expressed at low concentrations but is present in the nucleus, cytoplasm and mitochondria. Despite the fact that RNase H1 mRNA is modestly abundant, the levels of the protein are low because of the presence of an upstream open reading frame (uOrf) that suppresses translation. RNase H1 contains a mitochondrial localization signal that results in its presence and activity in mitochondria. If this signal peptide is deleted, the protein no longer localizes to the mitochondria.

Although it has been known for some time that RNaseH2 plays a significant role in DNA replication by degrading the RNA primers used to initiate DNA synthesis and removal of misincorporated ribonucleotides from DNA and that RNase H1 is essential for the RNA degradation induced by DNA-like antisense drugs, the biological roles of RNase H1 have been enigmatic, particularly in somatic cells. A previous attempt to create a constitutive knockout...
of RNase H1 in mice resulted in embryonic lethality, but did provide insights into several potential roles in mammalian cells. Embryonic death appeared to be the result of massive apoptosis secondary to mitochondrial dysfunction including reduction of mitochondrial DNA, suggesting that RNase H1 is necessary for a mitochondrial DNA replication. This suggestion was confirmed and extended in humans displaying mutations in RNase H1. These mutations impair mitochondrial DNA synthesis and result in adult onset mitochondrial encephalomyopathy.

To better understand the roles of RNase H1 in somatic cells, we sought to create viable liver specific knockout of RNase H1 in mice. We took two approaches. We constructed liver specific constitutive knockout mice by coupling expression of the Cre recombinase to the albumin promoter which is activated late in gestation in mice. We also established liver specific tamoxifen-inducible RNase H1 knockout mice. Both approaches yielded viable offspring that supported an evaluation of the roles of RNase H1 in hepatocytes and its roles in maintaining normal liver function. These systems were also important as final proof of RNase H1 mediated cleavage of target RNAs.

In both the constitutive and inducible knockout mice, the effects of loss of RNase H1 on hepatocytes and liver function were similar. The primary factor that resulted in damage to hepatocytes and the liver was the loss of RNase H1’s role in removing R Loops that occur during transcription of mitochondrial DNA. Attempts to maintain homeostasis despite the inability to clear R Loops resulted initially in increased mitochondrial DNA replication, fission, and fusion, but eventually hepatocyte apoptosis ensued resulting in severe liver toxicity.

Fortuitously, in the constitutive knockout mice, a clone of hepatocytes lost the Cre recombinase, so we were able to monitor recovery from the lesions produced by the RNase H1 knockout as the liver was repopulated with RNase H1 containing hepatocytes. Normal liver function was observed when only a fraction of the total hepatic RNase H1 activity was recovered confirming that there are substantially more hepatocytes than the minimum needed to maintain liver function in a normal animal. Both systems resulted in almost total loss of antisense drug activity, confirming that in hepatocytes, only RNase H1 is involved in the activity of the DNA-like antisense drugs.

Discussion

Establishment of viable constitutive and inducible liver specific RNase H1 knockout mice has enabled a significant advance in the understanding of the roles of mammalian RNase H1 in hepatocytes. It is now clear that RNase H1 is essential for mitochondrial function. It is also clear that RNase H1 plays an important role in nuclear functions. Although RNase H1 may play a meaningful role in DNA replication, our data suggest that its primary role is in assuring that transcription of GC rich regions of DNA proceeds successfully. Certainly, in the nucleus this important function is redundantly managed by topoisomerase1 and RNase H1 and in the mitochondria we suspect there is redundancy as well.

Of course, there are questions that remain to be answered. One of the most intriguing is what, if anything, are the roles of RNase H1 in the cytoplasm. We know it’s there and we know it is active. RNase H1 has two functional translation initiation sites, one that translates the entire protein including the mitochondrial localization signal, and a second that results in a protein without the localization signal, so evolution has assured that there is a possibility of translating a cytoplasmically localized protein (as well as nuclear). So how much of the shorter protein is made, what are the regulatory processes that might shift the translation initiation site? Is RNase H1 in the cytoplasm merely in transit, or does it play a meaningful role there? These are some of the interesting questions that remain.

Acknowledgment

Our sincere appreciation goes to Donna Parrett for her outstanding and efficient support.

Conflict of Interest

All authors are employed by Ionis Pharmaceuticals, Inc.

Funding

All research was funded by Ionis Pharmaceuticals, Inc.

References

1. Ceriello SM, Frolova EG, Feng C, Grinberg A, Love PE, Crouch RJ. Failure to produce mitochondrial DNA results in embryonic lethality in Rnaseh1 null mice. Mol Cell. 2003; 11:807-815.
2. Lima WF, Wu H, Crooke ST. The RNase H Mechanism. In Antisense Drug Technology: Principles, Strategies, and Applications, Second Edition, Crooke ST. (ed) Baco Raton FL: CRC Press, Chapter 2, 47-74, 2008.
3. Crouch RJ, Dirkson ML, Ribonuclease H. In: Nucleases. Linn SM and Robert RJ (eds). NY: Cold Spring Harbor Laboratory Press, 211–241, 1982.
4. Wu H, Lima WF, Crooke ST. Investigating the structure of human RNase H1 by site-directed mutagenesis. J Biol Chem. 2001; 276:23547–23553.
5. Nakamura H, et al. How does RNase H recognize a DNA-RNA hybrid? Proc Natl Acad Sci USA. 1991; 88:11535–11539.
6. Lima WF, Wu H, Nichols JG, Prakash TP, Ravikumar V, Crooke ST. Human RNase H1 uses one tryptophan and two lysines to position the enzyme at the 3-DNA/5-RNA terminus of the heteroduplex substrate. J Biol Chem. 2003; 278:49860–49867.
7. Wu H, Lima WF, Zhang H, Fan A, Sun H, Crooke ST. Determination of the role of the human RNase H1 in the pharmacology of DNA-like antisense drugs. J Biol Chem. 2004; 279:17181–17189.
8. Liang XH, Shen W, Sun H, Migauwa MT, Vickers TA, Crooke ST. Translation efficiency of mRNAs is increased by antisense oligonucleotides targeting upstream open reading frames. Nature Biotech. 2016; 34:875-880.
9. Hiller B, Achleitner M, Glage S, Naumann R, Behrendt R, Roers A. Mammalian RNase H2 removes ribonucleotides from DNA to maintain genome integrity. J Exp Med. 2012; 209:1419-1426.

10. Reyes A, Melchionda L, Nasca A, Carrara F, Lamantea E, Zanolini A, et al. RNase H1 mutations impair mtDNA replication and cause adult-onset mitochondrial encephalomyopathy. Am J Hum Genet. 2015; 97:186–193.

11. Lima WF, Murray HM, Damle SS, Hart CE, Hung G, De Hoyos CL, et al. Viable RNaseH1 knockout mice show RNaseH1 is essential for R loop processing, mitochondrial and liver function. Nucleic Acid Res. 2016; 44:5299-5312.

12. ElHage A, Webb S, Kerr and Tollervey D. Plos Genetics. 2014; 10:e1004716.

13. Li X, Manley JL. Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability. Cell. 2005; 122:365-378.