Genes & Diseases (2020) 7, 199–204

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

REVIEW ARTICLE

RISC assembly and post-transcriptional gene regulation in Hepatocellular Carcinoma

Prasanna K. Santhekadur*, Divya P. Kumar

Department of Biochemistry, Center of Excellence in Molecular Biology & Regenerative Medicine, JSS Medical College, JSS Academy of Higher Education and Research, Mysore, India

Received 30 July 2019; received in revised form 7 September 2019; accepted 9 September 2019
Available online 17 September 2019

KEYWORDS
Inflammation;
miRNA;
Oncogenes;
Regulation;
Tumor suppressor genes

Abstract RNA-induced silencing complex (RISC) is one of the basic eukaryotic cellular machinery which plays a pivotal role in post-transcriptional gene regulation. Discovery of miRNAs and their role in gene regulation have changed the course of modern biology. The method of gene silencing using small interfering RNAs and miRNAs has become major tool in molecular biology and genetic engineering. Hepatocellular Carcinoma (HCC) is a very common malignancy of liver in developing countries and due to various risk factors; the prevalence of this disease is rapidly increasing throughout the globe. There exists an imbalance in interplay between oncogenes and tumor suppressor genes and their regulation plays a major role in HCC growth, development and metastasis. The regulatory function of RISC and miRNAs make them a very important mediators of cancer signaling in HCC. Therefore, targeting the RISC complex for HCC therapy is the need of the time.

Introduction

The RNA-induced silencing complex is a large assembly of RNA binding multi protein complex in eukaryotic cells and it plays a pivotal role in gene expression and regulation.[1] The total molecular weight of this complex varies from 200 to 400 kDa depending on the stage of the cell cycle, nature of the cells, tissues and organs.[2] Some of these proteins are highly conserved in bacteria, yeast, plants and mammals.[3] The main function of this complex is degradation or suppression of various target specific mRNAs and regulation of protein synthesis at RNA level.[4] The regulatory property of this assembly is exploited in the studies of target specific gene knock out and in the generation of transgenic animals.[5] RISC, is a multiprotein assembly or it's a group of protein complex involving ribonucleoproteins which incorporates one strand of a single-stranded RNA (ss-RNA)

* Corresponding author. Department of Biochemistry, Center of Excellence in Molecular Biology and Regenerative Medicine, JSS Medical College, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagar, Mysore 570015, Karnataka, India.
E-mail address: prasannakumars@jssuni.edu.in (P.K. Santhekadur).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2019.09.009

fragment, such as various microRNAs (mi-RNAs), or double-stranded small interfering RNAs (commercially available artificial si-RNAs) and this phenomenon is very commonly known as RNA interference.[2,4] This landmark discovery was first reported by two scientists, Andrew Fire and Craig C. Mello from the United States which fetched them 2006 Nobel Prize for Physiology and Medicine.[6]

Components of RISC complex

RISC is a multiprotein complex and includes proteins such as Argonaute RISC Catalytic Component 2 (Ago2), Staphylococcal Nuclease Domain-Containing Protein 1 (SND1), Astrocyte Elevated Gene-1 (AEG-1), Fragile X Mental Retardation 1 (FMR1), VIG (vasa intronic gene), R2D2 (a dsRNA binding protein with two dsRNA binding domains), Aubergine (an Ago family protein) and Armitage-RNA helicase1.[7–9] The schematic representation of the RISC complex can be seen in (Fig. 1) Each component of this complex is very essential and important for proper synergistic functioning in *in vivo* or *in vitro* gene silencing.[1,7,8] It is a very well-known fact that eukaryotic or human cell contains thousands of miRNAs.[10] The expression and regulation of these miRNAs are also known to control pathophysiology of each individual cell, tissue or organ or animal or human beings via RISC complex.[11] Therefore, along with the expression of these miRNAs, the expression of each individual components of this complex itself controls the function of each cells, organs and complete multicellular organism itself including mammals and human beings.[1,6] Thus, posttranscriptional gene regulation and silencing by these RISC proteins plays a vital role in controlling various diseases and human health.[7] In this review article we are shedding torch light on the role of RISC complex in Hepatocellular Carcinoma.

Hepatocellular Carcinoma

Hepatocellular Carcinoma is one of the deadliest cancers and fourth most leading cause of cancer related mortality throughout the globe.[12] Although there are various risk factors associated and known to cause the development and progression of HCC, there are rapidly growing evidences showing that obesity and nonalcoholic fatty liver disease is becoming one of the leading causes for the HCC globally including India.[13–15] The other main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), environmental toxins like aflatoxins, smoking, heavy alcohol consumption, type 2 diabetes (T2D), obesity and genetic and heredity disorders.[13]

miRNA associated with Hepatocellular Carcinoma

There are many miRNAs that directly or indirectly associate with the development of HCC.[7,16] Some of the very important miRNAs which are involved in HCC are miR-21, miR-25, miR-155-5p, miR-210, miR-221 and miR-1246 etc.[17,18] The advanced and sophisticated research in miRNAs will lead to the elucidation of novel and relevant functional mechanisms which contributes to the development of cancer therapeutics. Thus, in this review we summarize the recent research developments in HCC associated miRNA biology. Many of these miRNAs have been

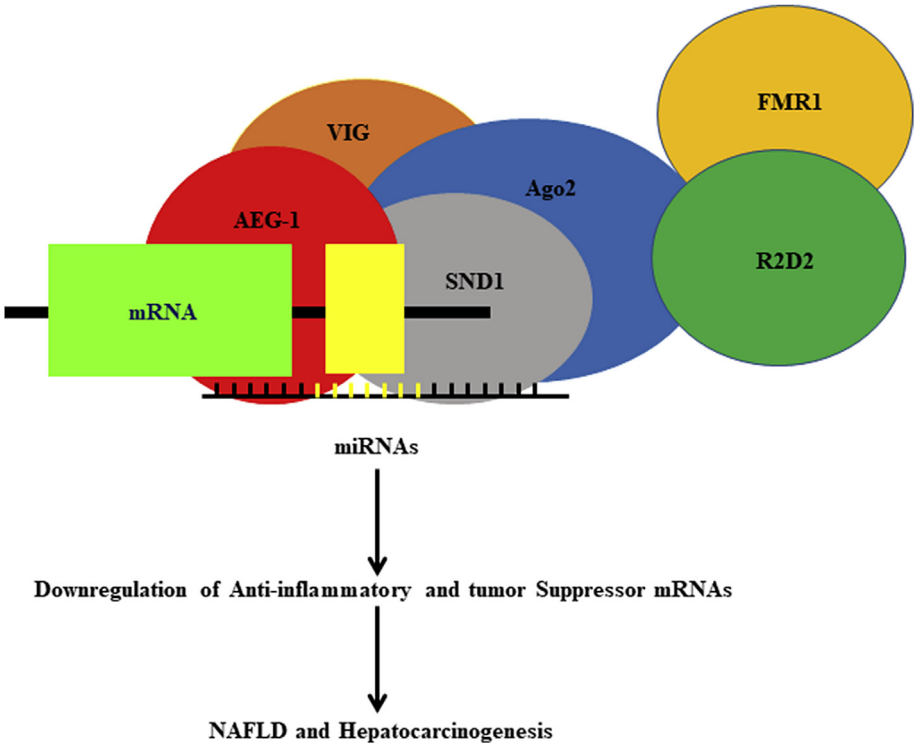

Figure 1 Schematic representation of RISC assembly and its role in post-transcriptional gene regulation.

found to be upregulated in HCC and few down regulated. The list of some of the important upregulated and down regulated miRNAs are mentioned in the (Table 1).[19–30] These miRNAs are mainly involved in cell cycle regulation, proliferation, apoptosis, autophagy, drug resistance, angiogenesis, epithelial-mesenchymal transition (EMT), migration, invasion, and metastasis. Therefore, these miRNAs controls and regulates almost all the hallmarks of cancers including the salient features of HCC.[16–18] In addition, some of these miRNAs can also be used as potential diagnostic and prediction markers in the detection of NAFLD and HCC.[17,26,29] Recently, there are many nucleic acid-based drugs available (siRNAs, miRNAs, long ncRNAs) and serve to have a beneficial and promising therapeutic potential for the treatment of HCC.[31]

Recently many studies have also showed that exosomes and exosomal miRNAs also play an important role in the development and progression in both NAFLD and HCC. Therefore, exosomal miRNAs also can be used as diagnostic tools and disease progression markers in HCC.[32,33]

miRNA associated with nonalcoholic fatty liver disease (NAFLD)

NAFLD is a chronic disease condition in which the accumulation of simple fats and lipids builds up in the liver that further progress to NASH (inflammation, damage of the hepatocytes of the liver) and finally to Hepatocellular Carcinoma.[14,27]

There are various factors and signaling pathways associated with the development of NAFLD and HCC. Recent studies also show that miRNA plays a major role in the development, progression and regulation of NAFLD.[7,14,16] The biogenesis and processing of miRNA and their function involves complex cascades of events.[1] One such cascade of events involves RISC units.[7] Therefore, RISC complex also plays a significant role in controlling the homeostasis, abundance and also functions of these miRNAs. Some of the very important miRNA which are associated with NALFD and HCC are miR-221, miR-21, miR-155, miR-33, etc.[7,17,18]

Gene regulation in HCC via RISC complex

The main components of the RISC assembly such as Ago2, SND1 and AEG-1 are known to function as multifaceted proteins in eukaryotic cells. Although they are involved in many functions, gene silencing is the major function of these protein. Due to this pivotal role all these proteins are also associated with the development of various cancers such as colon cancer, neuroblastoma, breast cancer, prostate cancer, pancreatic cancer and lung cancer.[1,7,34,35] They also play very significant role in the development, progression and metastasis of Hepatocellular Carcinoma. The overexpression of these proteins increases the RISC activity and HCC initiation, development and metastasis. Decreased expression of these proteins results in inhibition of tumor growth by increasing the expression of various tumor suppressor genes.[7] Therefore, the overexpression of these genes will activate various oncogenic signaling pathways. The Fig. 2 clearly shows the molecular mechanisms of gene regulation via RISC complex in HCC cells and is becoming a novel target for HCC therapy. Some of the important tumor suppressor genes known to be regulated by the RISC components are Phosphatase and tensin homolog (PTEN), sprouty RTK signaling antagonist 2 (SPRY2), transforming growth factor beta receptor 2 (TGFBR2), cyclin dependent kinase inhibitor 1A (CDKN1A) and cyclin dependent kinase inhibitor 1C (CDKN1C). These tumor suppressors are very important cell cycle regulators and growth factors. PTEN plays a major role in inhibition of protein synthesis during cancer growth. Transforming growth factor beta receptor 2 (TGFBR2) plays a role in induced or spontaneous development of Hepatocellular Carcinoma (HCC), liver inflammation, and fibrosis. AEG-1 is known to cause HCC initiation, growth, development and

Table 1 The list of miRNAs which causes NAFLD and HCC.

SL No	Micro RNA	Functions (references)
1)	miR-221	Aids in proliferation, migration, Angiogenesis in HCC.[16]
2)	miR-21	Promotes proliferation in HCC.[16–18]
3)	miR-34a	Steatosis and inflammation.[19]
4)	miR-155	Involved in tumorigenesis of HCC.[20]
5)	miR-122	Stimulates the expression of 24 hepatocytes-specific genes, including hepatocyte nuclear factor 6 (HNF6).[21]
6)	miR-192	Liver fibrogenesis.[22]
7)	miR-375	Steatosis and inflammation also reported as tumor suppressor by inhibiting AEG-1 expression.[23]
8)	miR-451	It is associated with NAFLD.[24]
9)	miR-301a	They play a role in HCC.[25]
10)	miR-197	Decreased in NAFLD.[26]
11)	miR-182	This is involved in NAFLD.[27]
12)	miR-29b-3p	This is involved in NAFLD.[28]
13)	miR-741-3p	This is involved in NAFLD.[28]
14)	miR-16	It is associated with NAFLD.[29]
15)	miR-146b	Decreased in NAFLD.[30]

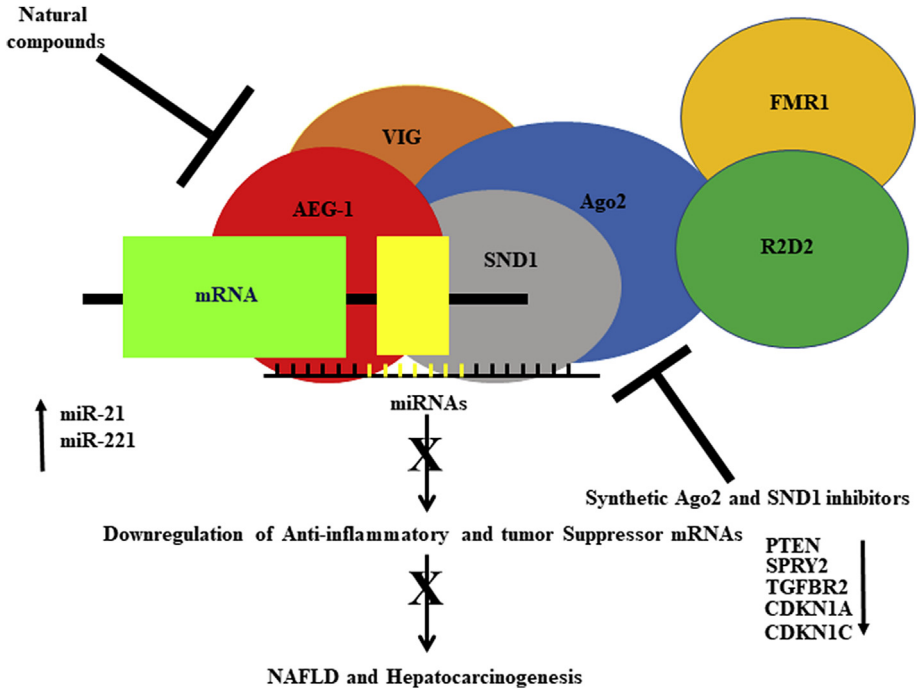

Figure 2 The possible mechanism of RNA-induced silencing complex and its components as novel targets for Hepatocellular Carcinoma therapy.

metastasis.[7,16] The SND1 is also known to cause almost all the hallmarks of cancer in HCC.[36] Argonaute2 also independently promotes tumor metastasis by up-regulating focal adhesion kinase transcription factor expression in HCC.[37] Therefore, these three RISC proteins independently act as oncogenes and also together in a complex their effect may increase synergistically in post transcriptional gene regulation.[1,17,37]

All these data from various studies clearly shows that RISC complex is a major player in cancer development, growth and metastasis in HCC. Various groups are already trying to target these proteins via various natural and synthetic compounds to design a potent drug for the highly aggressive Hepatocellular Carcinoma.

Conclusion

The RNA-induced silencing complex is an extremely versatile basic and vital cellular regulatory machine of almost all the eukaryotic cells. This complex can be loaded with numerous naturally occurring guide RNA or *in vitro* man-made artificial siRNA or miRNA of any desired sequence and this sequence specific RNA can be adapted to serve various knock down and knockout studies.[1,6]

A major challenge in the past decade or past century was to determine how many proteins are present in RISC complex and what are the different types of RISC components expressed in different eukaryotic cells, especially in human cells and the specific biochemical activities of each component of this complex.[1,7] Various cellular, molecular, biochemical, genetic and proteomic approaches have helped in identifying numerous components of the RISC complex.[38] It has also helped in studying individual protein of choice within this RISC complex. It is also known that proteomic approaches have helped in identifying the number of Argonaute family associated proteins.[39–41] Recent studies elucidated the numerous biochemical roles of these proteins in cancer biology along with their function in RISC assembly.[39,42] These findings also revealed that few of the very well-known oncogenes also are associated with RISC assembly.[7,16] This complex protein–protein interaction is playing a major role in posttranscriptional gene regulation especially in cancer. Here we have mainly focused on the function of these different components in reference to Hepatocellular Carcinoma.

It is possible to target this complex by gaining knowledge of minute details of the individual proteins along with th0rough scientific understanding and its molecular mechanism. A clear bird view of how post-transcriptional and post-translational modifications by this assembly controls tumor initiation, growth, development and metastasis via RISC function will also be very critical. Along with their role in post transcriptional modification, these individual proteins themselves undergo posttranslational modification. Recent studies have shown that human AEG1, SND1, AGO2 also undergo phosphorylation cell and tissue specific manner.[43–45] The post translational modification of these individual components plays a pivotal role in the RISC complex function. Sometime these modifications regulate and plays major and important contribution to form RISC complex and also help in their localization either in cytoplasm, nucleus, cell membranes or p-bodies. Due to these properties it is easy to target individual proteins in cell and tissue specific manner. The Ago2 and SND1 both have nuclease function and designing the inhibitors for these nuclease enzymes and targeting them with absolute specificity will be simple and effortless. Therefore, we can easily predict, and it is

tempting to speculate that RISC assembly is a suitable novel target for cancer therapy especially Hepatocellular Carcinoma.

Conflicts of interest

Both the authors have none to declare.

Acknowledgements

This work was supported by Ramalingaswami Re-entry fellowship, Department of Biotechnology (DBT), Govt. of India to both the authors. We are thankful to the Vice-Canceller of JSS AHER, Principal and Vice Principals of JSS Medical College.

References

1. Caudy AA, Ketting RF, Hammond SM, et al. A micrococcal nuclease homologue in RNAi effector complexes. *Nature*. 2003 Sep 25;425(6956):411—414.
2. van den Berg A, Mols J, Han J. RISC-target interaction: cleavage and translational suppression. *Biochim Biophys Acta*. 2008 Nov;1779(11):668—677.
3. Lingel A, Izaurralde E. RNAi: finding the elusive endonuclease. *RNA*. 2004 Nov;10(11):1675—1679.
4. Filipowicz W. RNAi: the nuts and bolts of the RISC machine. *Cell*. 2005 Jul 15;122(1):17—20.
5. Peng S, York JP, Zhang P. A transgenic approach for RNA interference-based genetic screening in mice. *Proc Natl Acad Sci U S A*. 2006 Feb 14;103(7):2252—2256.
6. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature*. 1998 Feb 19; 391(6669):806—811.
7. Yoo BK, Santhekadur PK, Gredler R, et al. Increased RNA-induced silencing complex (RISC) activity contributes to hepatocellular carcinoma. *Hepatology*. 2011 May;53(5): 1538—1548.
8. Caudy AA, Myers M, Hannon GJ, Hammond SM. Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev*. 2002 Oct 1;16(19):2491—2496.
9. Sontheimer EJ. Assembly and function of RNA silencing complexes. *Nat Rev Mol Cell Biol*. 2005 Feb;6(2):127—138.
10. Zhang R, Su B. Small but influential: the role of microRNAs on gene regulatory network and 3'UTR evolution. *J Genet Genomics*. 2009 Jan;36(1):1—6.
11. Witwer KW, Halushka MK. Toward the promise of microRNAs — Enhancing reproducibility and rigor in microRNA research. *RNA Biol*. 2016 Nov;13(11):1103—1116.
12. Yang JD, Roberts LR. Hepatocellular carcinoma: a global view. *Nat Rev Gastroenterol Hepatol*. 2010 Aug;7(8):448—458.
13. Yoo BK, Emdad L, Su ZZ, et al. Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. *J Clin Investig*. 2009 Mar;119(3):465—477.
14. Asgharpour A, Cazanave SC, Pacana T, et al. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. *J Hepatol*. 2016 Sep;65(3):579—588.
15. Kumar A, Acharya SK, Singh SP, et al. The Indian national association for study of the liver (INASL) consensus on prevention, diagnosis and management of hepatocellular carcinoma in India: the puri recommendations. *J Clin Exp Hepatol*. 2014 Aug;4(Suppl 3):S3—S26.
16. Santhekadur PK, Das SK, Gredler R, et al. Multifunction protein staphylococcal nuclease domain containing 1 (SND1) promotes tumor angiogenesis in human hepatocellular carcinoma through novel pathway that involves nuclear factor κB and miR-221. *J Biol Chem*. 2012;287(17):13952—13958.
17. Xu X, Tao Y, Shan L, et al. The role of MicroRNAs in hepatocellular carcinoma. *J Cancer*. 2018 Sep 8;9(19):3557—3569.
18. Pascut D, Krmac H, Gilardi F, et al. A comparative characterization of the circulating miRNome in whole blood and serum of HCC patients. *Sci Rep*. 2019 Jun 4;9(1):8265.
19. Ding J, Li M, Wan X, et al. Effect of miR-34a in regulating steatosis by targeting PPARα expression in nonalcoholic fatty liver disease. *Sci Rep*. 2015 Sep 2;5:13729.
20. Li DP, Fan J, Wu YJ, Xie YF, Zha JM, Zhou XM. MiR-155 up-regulated by TGF-β promotes epithelial-mesenchymal transition, invasion and metastasis of human hepatocellular carcinoma cells in vitro. *Am J Transl Res*. 2017 Jun 15;9(6): 2956—2965.
21. Laudadio I, Manfroid I, Achouri Y, et al. A feedback loop between the liver-enriched transcription factor network and miR-122 controls hepatocyte differentiation. *Gastroenterology*. 2012;142:119—129.
22. Ezhilarasan D. Role of MicroRNAs in hepatic fibrosis progression. *J Appl Pharm Sci*. 2018;8(05):174—178.
23. He XX, Chang Y, Meng FY, et al. MicroRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth in vitro and in vivo. *Oncogene*. 2012 Jul 12;31(28): 3357—3369.
24. Yamada H, Suzuki K, Ichino N, et al. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. *Clin Chim Acta*. 2013 Sep 23;424:99—103.
25. Guo Y, Xiong Y, Sheng Q, Zhao S, Wattacheril J, Flynn CR. A micro-RNA expression signature for human NAFLD progression. *J Gastroenterol*. 2016 Oct;51(10):1022—1030.
26. Celikbilek M, Baskol M, Taheri S, et al. Circulating microRNAs in patients with non-alcoholic fatty liver disease. *World J Hepatol*. 2014 Aug 27;6(8):613—620.
27. Tessitore A, Cicciarelli G, Del Vecchio F, et al. MicroRNA expression analysis in high fat diet-induced NAFLD-NASH-HCC progression: study on C57BL/6J mice. *BMC Cancer*. 2016 Jan 5; 16:3.
28. Nie J, Li CP, Li JH, Chen X, Zhong X. Analysis of non-alcoholic fatty liver disease microRNA expression spectra in rat liver tissues. *Mol Med Rep*. 2018 Sep;18(3):2669—2680.
29. Mehta R, Otgonsuren M, Younoszai Z, Allawi H, Raybuck B, Younossi Z. Circulating miRNA in patients with non-alcoholic fatty liver disease and coronary artery disease. *BMJ Open Gastroenterol*. 2016 Jul 26;3(1):e000096.
30. He S, Guo W, Deng F, et al. Targeted delivery of microRNA 146b mimic to hepatocytes by lactosylated PDMAEMA nanoparticles for the treatment of NAFLD. *Artif Cells Nanomed Biotechnol*. 2018;46(suppl 2):217—228.
31. Lieberman J. Tapping the RNA world for therapeutics. *Nat Struct Mol Biol*. 2018 May;25(5):357—364.
32. Wang S, Wang JQ, Lv XW. Exosomal miRNAs as biomarkers in the diagnosis of liver disease. *Biomark Med*. 2017 May;11(6): 491—501.
33. Dongiovanni P, Meroni M, Longo M, Fargion S, Fracanzani AL. miRNA signature in NAFLD: a turning point for a non-invasive diagnosis. *Int J Mol Sci*. 2018;19:3966.
34. Wang N, Du X, Zang L, et al. Prognostic impact of Metadherin-SND1 interaction in colon cancer. *Mol Biol Rep*. 2012 Dec; 39(12):10497—10504.
35. Emdad L, Janjic A, Alzubi MA, et al. Suppression of miR-184 in malignant gliomas upregulates SND1 and promotes tumor aggressiveness. *Neuro Oncol*. 2015 Mar;17(3):419—429.
36. Santhekadur PK, Akiel M, Emdad L, et al. Staphylococcal nuclease domain containing-1 (SND1) promotes migration and

invasion via angiotensin II type 1 receptor (AT1R) and TGFβ signaling. *FEBS Open Bio*. 2014 Apr 1;4:353—361.
37. Cheng N, Li Y, Han ZG. Argonaute2 promotes tumor metastasis by way of up-regulating focal adhesion kinase expression in hepatocellular carcinoma. *Hepatology*. 2013 May;57(5): 1906—1918.
38. Caudy AA, Hannon GJ. Induction and biochemical purification of RNA-induced silencing complex from Drosophila S2 cells. *Methods Mol Biol*. 2004;265:59—72.
39. Höck J, Weinmann L, Ender C, et al. Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. *EMBO Rep*. 2007 Nov;8(11): 1052—1060.
40. Bühler M, Verdel A, Moazed D. Tethering RITS to a nascent transcript initiates RNAi- and heterochromatin-dependent gene silencing. *Cell*. 2006 Jun 2;125(5):873—886.
41. Pratt AJ, MacRae IJ. The RNA-induced silencing complex: a versatile gene-silencing machine. *J Biol Chem*. 2009 Jul 3; 284(27):17897—17901.
42. Jariwala N, Rajasekaran D, Mendoza RG, et al. Oncogenic role of SND1 in development and progression of hepatocellular carcinoma. *Cancer Res*. 2017 Jun 15;77(12):3306—3316.
43. Krishnan RK, Nolte H, Sun T, et al. Quantitative analysis of the TNF-α-induced phosphoproteome reveals AEG-1/MTDH/LYRIC as an IKKβ substrate. *Nat Commun*. 2015 Apr 7;6:6658.
44. Su C, Gao X, Yang W, et al. Phosphorylation of Tudor-SN, a novel substrate of JNK, is involved in the efficient recruitment of Tudor-SN into stress granules. *Biochim Biophys Acta Mol Cell Res*. 2017 Mar;1864(3):562—571.
45. Golden RJ, Chen B, Li T, et al. An Argonaute phosphorylation cycle promotes microRNA-mediated silencing. *Nature*. 2017 Feb 9;542(7640):197—202.