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Review Article

Telomerase, an Accomplice of RNA-induced Silencing Complex in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most lethal and widespread cancers in the human race. Despite its fatal attributes, there is only a limited understanding of the factors contributing to its pathogenesis at the cellular and molecular levels. Consequently, unraveling new facets involved in HCC progression is elemental for establishing novel targets and biomarkers for this disease. Over the last few years, emerging evidence signifies the role of RNA-induced silencing complex in mediating gene silencing and contributing to HCC. Recent studies also highlight the importance of human telomerase holoenzyme and its complex accessory proteins in the development of HCC. The current review encompasses the multifaceted roles of RNA-induced silencing complex and telomerase activity as well as their synergistic function in HCC.

Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor known for its heterogeneity owing to several factors associated with its development. Viral infection, aflatoxin, alcoholism, nonalcoholic steatohepatitis, smoking, and, in rare cases, genetic conditions are the main factors contributing to HCC. It is the fourth most common cause of cancer-related death worldwide[1] and affects all segments of the human population. The substantial variation in HCC incidence reflects the prevalence of specific etiological factors as well as the effects of ethnicity. The occurrence of HCC in developing regions like Asia and Africa contributes to viral hepatitis. On the contrary, diet-associated conditions like fatty liver diseases are said to be the driving factor of HCC in developed regions.[2] The pathogenesis of HCC is not completely deciphered; thus, early diagnosis and treatment are not always possible.

Several theories have been put forward to understand the progression of HCC; of these, microRNA (miRNA)-based regulatory mechanisms mainly involving gene silencing have been an important focus of research in molecular biology.[3] In particular, RNA interference (RNAi) is a dynamic operation of gene silencing that influences several facets of eukaryotic biology. At the molecular level, RNA-induced silencing complex (RISC), a ribonucleoprotein complex, governs RNAi.[4] The programming of RISC to mark relevant RNAs and execute gene silencing has favored its potential as an effective therapeutic target for HCC.

Another approach to explain the underlying principles of HCC progression reflects on telomere biology as a potential target for hepatocarcinogenesis.[5] Telomere shortening and telomerase reactivation are hallmarks of HCC.[6] Telomerase reactivation in HCC maintains the telomere functionality for the continuous proliferation of cancer cells and the development of carcinoma. Thus, targeting telomerase could also be a promising approach to control the progression of HCC.

Owing to the current limitations of treatment options for HCC, it is important to identify novel therapeutic targets. This review highlights the alliance between two key cellular pathways, RISC and telomerase, which could be a promising molecular approach for tackling HCC.

RISC–a multifaceted gene-silencing composite

RISC is a heterogeneous ribonucleoprotein complex in eukaryotic cells involved in RNAi. In 2006, two eminent scientists, Andrew

Keywords: Hepatocellular carcinoma; RNA-induced silencing complex; Gene silencing; Telomerase; Immortality.

Abbreviations: AEG1, astrocyte elevated gene 1; AGO2, agronuate 2; HCC, hepatocellular carcinoma; miRNA, microRNA; RISC, RNA-induced silencing complex; RNAi, RNA interference; SND1, Staphylococcal nuclease and Tudor domain-containing 1; TERC, telomerase RNA component; TERRA, telomeric repeat-containing RNA; TERT, telomerase reverse transcriptase protein.

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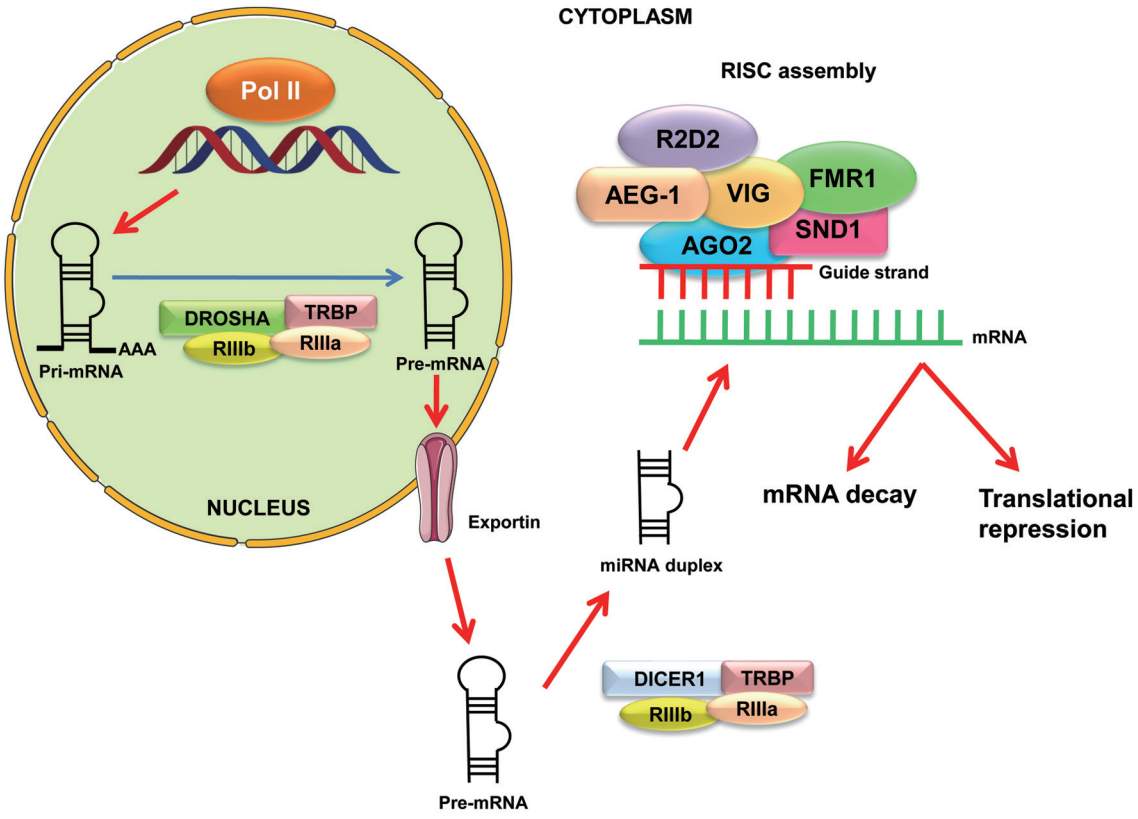

Fig. 1. Schematic representation of miRNA biogenesis and RISC assembly. miRNAs play an important role in the regulation of genes. Primary miRNAs are cleaved in the nucleus to generate precursor miRNAs. Furthermore, precursor miRNAs undergo processing in the cytoplasm to form a miRNA duplex. RISC, via its catalytic AGO2 or SND1, specifically incorporates the guide strand, which is base paired with the target mRNA, leading to gene silencing. AGO2, argonaute2; miRNAs, microRNAs; mRNA, messenger RNA; RISC, RNA-induced silencing complex; SND1, Staphylococcal nuclease and Tudor domain-containing 1.

Fire, and Craig C. Mellow, shared the Noble Prize in Physiology or Medicine for their landmark work on RNAi in nematodes, which was published in 1998.[7] Shortly after this discovery, Gregory Hannon and his colleagues identified the components of the RISC machinery at the Cold Spring Harbor Laboratory, New York, USA.[8] Later, at Virginia Commonwealth University, Richmond, Virginia, USA, Devanand Sarkar and his coworkers Prasanna K. Santhekadur and Byoung Kwon Yoo identified a novel constituent of this regulatory complex, called astrocyte elevated gene1 (AEG1). They elucidated the RISC activity using HCC cells and showed the major roles of Staphylococcal nuclease and Tudor domain-containing 1 (SND1) and AEG1 in RISC activity.[9]

The molecular weight of RISC ranges from 200 kDa to 400 kDa, depending on the characteristics of the cells, tissues, and organs as well as the phase of the cell cycle.[10] Broadly, the RISC machinery is triggered by the occurrence of double-stranded RNA in the eukaryotic cytoplasm, which is then modified into compact regulatory RNAs (20–30 nucleotides in length).[4] Argonaute protein, a prominent protein of RISC, aids in the recruitment of guide miRNA to the 3′-untranslated region of target mRNA and regulates transcript expression through RISC-mediated mRNA cleavage (Fig. 1).[9,11,12]

In the aftermath of target recognition, several patterns of RISC effector processes are exhibited. In one such approach, RISC subjects its complementary mRNA to slicing or hydrolysis by cellular exonucleases, which obstructs protein encoding.[13] In the mammalian system, the mode of gene silencing by RISC is ubiquitously through translational repression guided by miRNAs.[14] In some cases, RISCs are also found to directly act on the genome.[15] Additionally, as governors of gene expression, miRNAs regulate an array of vital cellular processes like proliferation, differentiation, apoptosis, *etc.*[16] These versatile miRNAs can serve as oncogenes or oncosuppressor genes, depending on their association with the target mRNAs.[17] Anomalous activation and inactivation of these genes are vital processes contributing to malignancy.[16] Therefore, understanding the operation of RISC at the molecular level could furnish a foundation for risk analysis, timely diagnosis, and successful treatment.

Telomerase–a catalyst of continuance

In October 2009, Elizabeth H. Blackburn, Carol W. Greider, and Jack W. Szostak were jointly awarded the Nobel Prize for Physiology or Medicine for their discovery of "how chromosomes are safeguarded by the telomeres and the enzyme telomerase".[18–20] Telomerase is composed of two core components: the telomerase RNA component (TERC) and the telomerase reverse transcriptase protein (TERT). TERT congregates with the intrinsic RNA template within the telomerase RNA component to recurrently synthesize tandem repeats of DNA at the telomeric region of the chromosome.[21] Telomeres are the repetitive end regions of the chromosomes that preserve genomic integrity. The cumulative shortening of telomeres in normal cells during consecutive

cell divisions promotes chromosomal instability and triggers cell death. In the vast majority of cells that gain oncogenic characteristics, cellular senescence is bypassed and the telomere length is preserved by telomerase.[22] This signifies that telomere-telomerase functionality is critical for cancer initiation and tumor endurance. The mechanisms underlying telomerase expression and telomere length maintenance are regulated by transcriptional, post-transcriptional, and epigenetic processes. A thorough understanding of these processes may offer novel biomarkers and targets for the timely diagnosis and prognosis of disease and the development of therapeutics.[23]

Significance of RISC in HCC

The regulatory function of RISC makes it a crucial mediator of cancer signaling in HCC.[8] The coordinated functioning of every constituent of this cellular machinery is vital for gene silencing. It is a very well-established fact that eukaryotic cells contain several miRNAs, which regulate the traits of an individual cell, tissues, organs, or organism as a whole via the RISC complex. In addition, the expression of each constituent of the complex has a key role in gene silencing.[10] Therefore, targeting this versatile gene silencing machinery for HCC therapy is indispensable.

Several miRNAs have been linked to the development of HCC, either directly or indirectly. Most of these are upregulated, while a few are downregulated. The chief regulatory mechanisms of miRNAs in HCC include proliferation, invasion, epithelial–mesenchymal transition, metastasis, drug resistance, apoptosis, angiogenesis, and autophagy.[10] Some important miRNAs associated with hepatocarcinogenesis are miR-21, miR-155-5p,miR-25, miR-221, miR-210, miR-1246, *etc.*[11,24] Furthermore, Drosha and DiGeorge syndrome critical region 8, the principle enzymes involved in the processing phase of primary miRNA to precursor miRNA in several types of cancer cells are constantly overexpressed in HCC.[15] To summarize, some of these miRNAs have the potential to be used as diagnostic and predictive markers for the detection of HCC.[25,26]

AEG1, also known as lyric, 3D3, and metadherin, plays a key role in carcinogenesis regulation.[27] AEG1 has been found to be overexpressed in a wide variety of cancers, including HCC. According to the analysis of cancer cell lines and a sizable group of patient cohorts, there are inverse statistical correlations between the AEG1 expression level and poor prognosis as well as decreased patient survival. In-depth studies also have shown that AEG1 controls various hallmarks of HCC development and provides resistance to therapeutic intervention.[28]

Argonaute 2 (AGO2) is a well-known component of RISC, playing an important role in gene expression. It is known to arbitrate tumor-promoting transcriptomic alterations during carcinogenesis, including HCC.[29] Of the four members of the argonaute protein family, AGO2 is characteristic of slicer activity in humans. It is important for the maturation of miRNAs and the gene-silencing mechanisms regulated by small RNA.[30,31] Studies have documented that upregulation of AGO2, a mediator of miRNA-guided gene silencing, regulates HCC progression by particularly enhancing the scope of oncogenic miRNAs (*e.g.,* miR-21) to suppress their target oncosuppressor genes (*e.g.,* phosphatase and tensin homolog).[32,33]

Interestingly, SND1 regulates lipid metabolism, inflammation, and other characteristic features of cancer by numerous processes, indicating that targeting SND1 might be an effective treatment option for HCC.[34] Studies have demonstrated that SND1 overexpression favors an underlying pro-inflammatory environment within the liver, which intensifies with age, creating favorable conditions for tumorigenesis.[35] SND1 has been shown to interact with a wide range of proteins, thereby regulating elemental cellular processes such as transcription, mRNA splicing, RNA editing, and miRNA activity. Available evidence supports the interaction of SND1 with monoglyceride lipase, which functions as an oncosuppressor in HCC. The interaction results in the proteasomal degradation of monoglyceride lipase, which favors SND1 to employ its protumorigenic activity.[36]

Telomere biology and HCC

Telomere shortening has been delineated as a principal feature of chronic liver diseases. This has fueled the postulation that telomere shortening contributes to genomic instability, which drives HCC. Furthermore, telomerase reactivation has been indicated to mediate HCC development. Also, shorter lengths of telomeres prevalent in HCCs compared to normal livers point to the activation of telomerase at a later stage of HCC progression. Collectively, the available evidence suggests that during the preliminary phases of hepatocarcinogenesis, telomere shortening leads to chromosomal instability and cancer-stimulating lesions. On the contrary, telomerase reactivation is essential for malignant progression in the later stages of hepatocarcinogenesis as it reinstates chromosomal stability to a degree suitable for cancer-cell viability.[37]

In HCC, the telomere length is maintained by several processes that trigger TERT expression. Aggressive HCC is characterized by long telomeres. TERT favors immortalization by preserving telomeres in cancer cells. Thus, mediators of TERT transcription are anticipated to be viable targets for HCC treatment. Several studies have supported the significance of TERT promoter mutations in liver carcinogenesis. These are the most prevalent mutations and earliest genetic events in HCC progression.[38,39] It has been found to be the most frequent in hepatitis C virus-related HCC and has been linked with elevated TERT levels and longer telomere lengths.[40,41] These findings are significant in terms of understanding the progression and prognosis of HCC.

Regardless of their heterochromatic feature, telomeres are transcribed to give rise to long noncoding RNAs called telomeric repeat-containing RNA (TERRA). The TERRA component is significant in telomere biology and in the regulation of telomerase activity. In HCC, both cellular and extracellular expression of TERRA are dysregulated.[42] This downregulation of TERRA considerably promotes HCC cell proliferation and metastasis by increasing telomerase activity and promoting telomere elongation.[43]

The alliance of telomerase and RISC in HCC

The functioning of human telomerase holoenzyme is regulated by a complex of accessory proteins that are responsible for the assembly, localization, and stability of the enzyme. Telomerase biology-related gene defects impair the regeneration of vital stem cell populations and result in clinical conditions like telomeropathies. Conversely, telomerase activation in somatic cells favors neoplastic cells to divide endlessly, thus promoting carcinogenesis. Recent studies have suggested that the RNAi machinery adds a new level of complexity to the action of human telomerase. RISC components like AGO2 and SND1 are known to play pivotal roles in TERC stability and function.[44–46]

The significance of AGO2 as an important mediator of hepatocarcinogenesis is well established. Through the formation of the RISC-loading complex, AGO2 exerts its role in the progres-

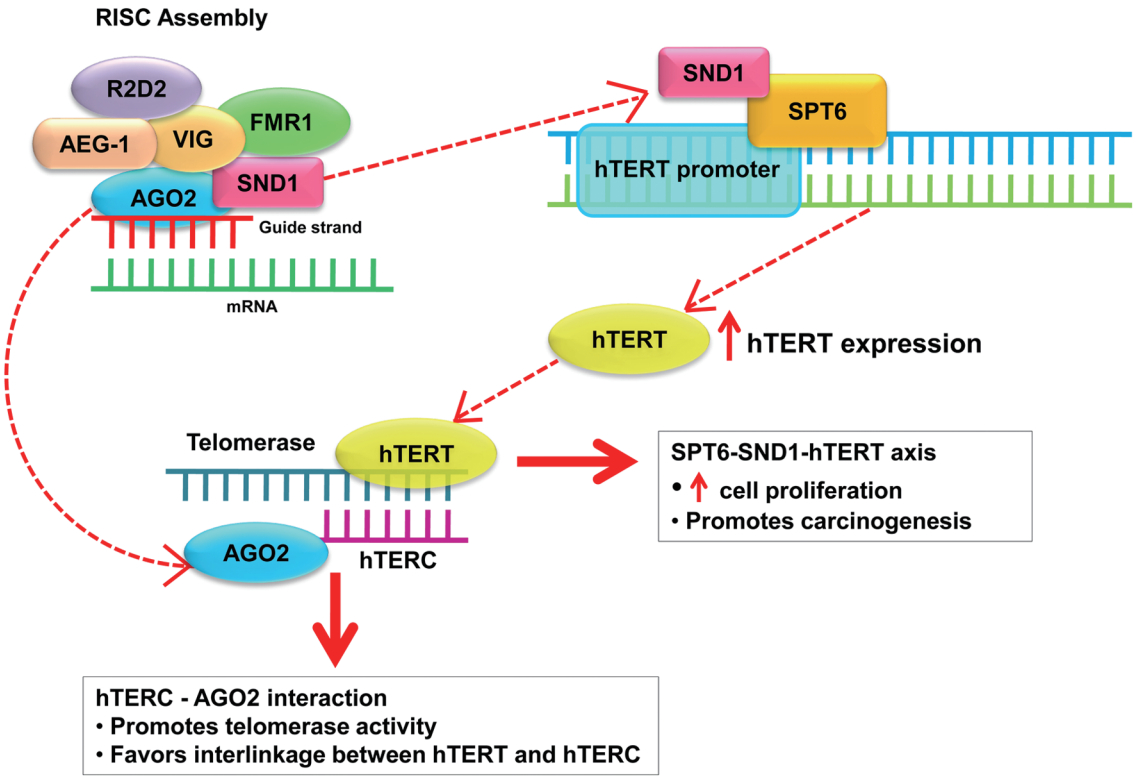

Fig. 2. Schematic representation of the interaction between RISC components and telomerase in promoting HCC. AGO2, a key component of RISC, binds to TERC, favors the interlinkage of TERC and TERT, and enhances telomerase activity. STP6, a transcription elongation factor, binds to the TERT promoter region and interacts with SND1, another key component of RISC to increase hTERT production and promote carcinogenesis. AGO2, argonaute2; HCC, hepatocellular carcinoma; RISC, RNA-induced silencing complex; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase protein; SND1, Staphylococcal nuclease and Tudor domain-containing 1.

sion of HCC. In addition, recent studies have highlighted AGO2 as an important TERC-binding protein. AGO2 primarily interacts with small RNA of TERC, originating from the 3′-end of TERC. Thus, any disruptions in the TERC regions that are complementary to the small RNA of TERC lead to an impaired association between TERC and AGO2.[44] AGO2 mediates telomerase activity and favors the interlinkage between TERT and TERC. AGO2 depletion results in shorter telomeres as well as decreased proliferation rates *in vitro* and *in vivo*, suggesting diminished telomerase activity.[45] Collectively, this lays the foundation for targeting the association between AGO2 and TERC to treat HCC.

SND1 is another key component of the RNAi machinery, whose role in the progression of liver carcinogenesis is well known. The significance of SND1 for the activity of human telomerase remains unexplored and demands further insights to understand its function in relation to telomerase activity. A study has demonstrated the role of SPT6 synergized with SND1-promoted colorectal cancer progression by targeting hTERT. Based on this evidence, an analysis can be put forward that inhibits the SPT6-SND1-hTERT axis, which may create a therapeutic vulnerability for HCC as well.[46]

There is no doubt that HCC is emerging at an alarming rate worldwide. The current treatment modalities have several shortcomings. HCC is an aggressive cancer that can be tackled only through improved therapeutic approaches. Accordingly, RISC has proven to be an effective therapeutic target. It can be loaded with a wide variety of naturally occurring guide RNA or *in-vitro* man-made artificial siRNA or miRNA and channeled for use in various knockdown and knockout studies. The post-translational gene regulation and silencing activity of RISC can be pivotal in controlling a wide array of diseases, including HCC. Thus, further exploration of this RNAi machinery and its molecular mechanism could be a viable treatment option for HCC. Furthermore, HCC progression is also adversely affected by the human telomerase holoenzyme machinery. Unraveling various mechanisms to prevent telomerase activation could further disrupt telomere functionality and HCC progression. Another promising approach could be targeting the components exerting a role in RISC functionality as well as telomerase activation. AGO2 and SND1 could be suitable targets from this perspective, as they could hinder the progression of HCC through multiple pathways (Fig. 2). In conclusion, targeting RISC and telomerase activity as well as unraveling the synergistic action of the two machineries could be important for the diagnosis and prognosis of HCC.

Conclusions

The prevalence of HCC is increasing globally, and it is one of the leading causes of cancer-related deaths worldwide. Thus, the identification of novel targets is crucial for improving treatment strategies. Our review highlights the synergistic role of RISC and telomerase in the progression of HCC and their potential as therapeutic targets for HCC. Further analysis of these regulatory pathways may be beneficial for the management of liver carcinomas.

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Conflict of interest

PKS has been an editorial board member of *Gene Expression* since August 2022. The authors have no other conflict of interests related to this publication.

Author contributions

Study design, acquisition of data, data analysis, drafting, and critical revision of the manuscript (LDP), data analysis and critical revision of the manuscript (VDS, NGSG, and SHK), study inception, concept design, data analysis, critical revision of the manuscript, and supervision (PKS).

References

[1] Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019;16(10):589–604. doi:10.1038/s41575-019-0186-y, PMID:31439937.

[2] Samant H, Amiri HS, Zibari GB. Addressing the worldwide hepatocellular carcinoma: epidemiology, prevention and management. J Gastrointest Oncol 2021;12(Suppl 2):S361–S373. doi:10.21037/jgo.2020.02.08, PMID:34422400.

[3] Chu R, Mo G, Duan Z, Huang M, Chang J, Li X, *et al*. miRNAs affect the development of hepatocellular carcinoma via dysregulation of their biogenesis and expression. Cell Commun Signal 2014;12:45. doi:10.1186/s12964-014-0045-y, PMID:25012758.

[4] Pratt AJ, MacRae IJ. The RNA-induced silencing complex: a versatile gene-silencing machine. J Biol Chem 2009;284(27):17897–17901. doi:10.1074/jbc.R900012200, PMID:19342379.

[5] Donaires FS, Scatena NF, Alves-Paiva RM, Podlevsky JD, Logeswaran D, Santana BA, *et al*. Telomere biology and telomerase mutations in cirrhotic patients with hepatocellular carcinoma. PLoS One 2017;12(8):e0183287. doi:10.1371/journal.pone.0183287, PMID:28813500.

[6] In der Stroth L, Tharehalli U, Günes C, Lechel A. Telomeres and Telomerase in the Development of Liver Cancer. Cancers (Basel) 2020;12(8):2048. doi:10.3390/cancers12082048, PMID:32722302.

[7] 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;391(6669):806–811. doi:10.1038/35888, PMID:9486653.

[8] Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 2000;404(6775):293–296. doi:10.1038/35005107, PMID:10749213.

[9] Santhekadur PK, Kumar DP. RISC assembly and post-transcriptional gene regulation in Hepatocellular Carcinoma. Genes Dis 2020;7(2):199–204. doi:10.1016/j.gendis.2019.09.009, PMID:32215289.

[10] Yoo BK, Santhekadur PK, Gredler R, Chen D, Emdad L, Bhutia S, *et al*. Increased RNA-induced silencing complex (RISC) activity contributes to hepatocellular carcinoma. Hepatology 2011;53(5):1538–1548. doi:10.1002/hep.24216, PMID:21520169.

[11] Xu X, Tao Y, Shan L, Chen R, Jiang H, Qian Z, *et al*. The Role of MicroRNAs in Hepatocellular Carcinoma. J Cancer 2018;9(19):3557–3569. doi:10.7150/jca.26350, PMID:30310513.

[12] Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer 2015;15(6):321–333. doi:10.1038/nrc3932, PMID:25998712.

[13] Tolia NH, Joshua-Tor L. Slicer and the argonautes. Nat Chem Biol 2007;3(1):36–43. doi:10.1038/nchembio848, PMID:17173028.

[14] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120(1):15–20. doi:10.1016/j.cell.2004.12.035, PMID:15652477.

[15] Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal SI, *et al*. RNAi-mediated targeting of heterochromatin by the RITS complex. Science 2004;303(5658):672–676. doi:10.1126/science.1093686, PMID:14704433.

[16] Chu R, Mo G, Duan Z, Huang M, Chang J, Li X, *et al*. miRNAs affect the development of hepatocellular carcinoma via dysregulation of their biogenesis and expression. Cell Commun Signal 2014;12:45. doi:10.1186/s12964-014-0045-y, PMID:25012758.

[17] Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther 2016;1:15004. doi:10.1038/sigtrans.2015.4, PMID:29263891.

[18] Szostak JW, Blackburn EH. Cloning yeast telomeres on linear plasmid vectors. Cell 1982;29(1):245–255. doi:10.1016/0092-8674(82)90109-x, PMID:6286143.

[19] Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985;43(2 Pt 1):405–413. doi:10.1016/0092-8674(85)90170-9, PMID:3907856.

[20] Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 1989;337(6205):331–337. doi:10.1038/337331a0, PMID:2463488.

[21] Dey A, Chakrabarti K. Current Perspectives of Telomerase Structure and Function in Eukaryotes with Emerging Views on Telomerase in Human Parasites. Int J Mol Sci 2018;19(2):333. doi:10.3390/ijms19020333, PMID:29364142.

[22] Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med 2016;8(1):69. doi:10.1186/s13073-016-0324-x, PMID:27323951.

[23] Akincilar SC, Unal B, Tergaonkar V. Reactivation of telomerase in cancer. Cell Mol Life Sci 2016;73(8):1659–1670. doi:10.1007/s00018-016-2146-9, PMID:26846696.

[24] Pascut D, Krmac H, Gilardi F, Patti R, Calligaris R, Crocè LS, *et al*. A comparative characterization of the circulating miRNome in whole blood and serum of HCC patients. Sci Rep 2019;9(1):8265. doi:10.1038/s41598-019-44580-x, PMID:31164669.

[25] Lieberman J. Tapping the RNA world for therapeutics. Nat Struct Mol Biol 2018;25(5):357–364. doi:10.1038/s41594-018-0054-4, PMID:29662218.

[26] Xie Y, Yao Q, Butt AM, Guo J, Tian Z, Bao X, *et al*. Expression profiling of serum microRNA-101 in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Cancer Biol Ther 2014;15(9):1248–1255. doi:10.4161/cbt.29688, PMID:24971953.

[27] Banerjee I, Fisher PB, Sarkar D. Astrocyte elevated gene-1 (AEG-1): A key driver of hepatocellular carcinoma (HCC). Adv Cancer Res 2021;152:329–381. doi:10.1016/bs.acr.2021.05.003, PMID:34353442.

[28] Robertson CL, Srivastava J, Rajasekaran D, Gredler R, Akiel MA, Jariwala N, *et al*. The role of AEG-1 in the development of liver cancer. Hepat Oncol 2015;2(3):303–312. doi:10.2217/hep.15.10, PMID:26798451.

[29] Zhang K, Pomyen Y, Barry AE, Martin SP, Khatib S, Knight L, *et al*. AGO2 Mediates *MYC* mRNA Stability in Hepatocellular Carcinoma. Mol Cancer Res 2020;18(4):612–622. doi:10.1158/1541-7786.MCR-19-0805, PMID:31941754.

[30] Schirle NT, MacRae IJ. The crystal structure of human Argonaute2. Science 2012;336(6084):1037–1040. doi:10.1126/science.1221551, PMID:22539551.

[31] Höck J, Meister G. The Argonaute protein family. Genome Biol 2008;9(2):210. doi:10.1186/gb-2008-9-2-210, PMID:18304383.

[32] Ye ZL, Huang Y, Li LF, Zhu HL, Gao HX, Liu H, *et al*. Argonaute 2 promotes angiogenesis via the PTEN/VEGF signaling pathway in human hepatocellular carcinoma. Acta Pharmacol Sin 2015;36(10):1237–

4125. doi:10.1038/aps.2015.18, PMID:25937637.
[33] Santhekadur PK. Ago2 is a Novel Target for Hepatocellular Carcinoma. Nov Appro Drug Des Dev 2019;5(2):555659. doi:10.19080/NAPDD.2019.05.555659.
[34] Chidambaranathan-Reghupaty S, Mendoza R, Fisher PB, Sarkar D. The multifaceted oncogene SND1 in cancer: focus on hepatocellular carcinoma. Hepatoma Res 2018;4:32. doi:10.20517/2394-5079.2018.34, PMID:32258418.
[35] Jariwala N, Rajasekaran D, Mendoza RG, Shen XN, Siddiq A, Akiel MA, *et al*. Oncogenic Role of SND1 in Development and Progression of Hepatocellular Carcinoma. Cancer Res 2017;77(12):3306–3316. doi:10.1158/0008-5472.CAN-17-0298, PMID:28428278.
[36] Rajasekaran D, Jariwala N, Mendoza RG, Robertson CL, Akiel MA, Dozmorov M, *et al*. Staphylococcal Nuclease and Tudor Domain Containing 1 (SND1 Protein) Promotes Hepatocarcinogenesis by Inhibiting Monoglyceride Lipase (MGLL). J Biol Chem 2016;291(20):10736–10746. doi:10.1074/jbc.M116.715359, PMID:26997225.
[37] Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 2006;6(9):674–687. doi:10.1038/nrc1934, PMID:16929323.
[38] Hirai M, Kinugasa H, Nouso K, Yamamoto S, Terasawa H, Onishi Y, *et al*. Prediction of the prognosis of advanced hepatocellular carcinoma by TERT promoter mutations in circulating tumor DNA. J Gastroenterol Hepatol 2021;36(4):1118–1125. doi:10.1111/jgh.15227, PMID:32830343.
[39] Lee SE, Chang SH, Kim WY, Lim SD, Kim WS, Hwang TS, *et al*. Frequent somatic TERT promoter mutations and CTNNB1 mutations in hepatocellular carcinoma. Oncotarget 2016;7(43):69267–69275. doi:10.18632/oncotarget.12121, PMID:27661004.
[40] Jang JW, Kim JS, Kim HS, Tak KY, Lee SK, Nam HC, *et al*. Significance of TERT Genetic Alterations and Telomere Length in Hepatocellular Carcinoma. Cancers (Basel) 2021;13(9):2160. doi:10.3390/cancers13092160, PMID:33946181.
[41] Kwa WT, Effendi K, Yamazaki K, Kubota N, Hatano M, Ueno A, *et al*. Telomerase reverse transcriptase (TERT) promoter mutation correlated with intratumoral heterogeneity in hepatocellular carcinoma. Pathol Int 2020;70(9):624–632. doi:10.1111/pin.12974, PMID:32559017.
[42] Manganelli M, Grossi I, Corsi J, D'Agostino VG, Jurikova K, Cusanelli E, *et al*. Expression of Cellular and Extracellular *TERRA*, *TERC* and *TERT* in Hepatocellular Carcinoma. Int J Mol Sci 2022;23(11):6183. doi:10.3390/ijms23116183, PMID:35682861.
[43] Cao H, Zhai Y, Ji X, Wang Y, Zhao J, Xing J, *et al*. Noncoding telomeric repeat-containing RNA inhibits the progression of hepatocellular carcinoma by regulating telomerase-mediated telomere length. Cancer Sci 2020;111(8):2789–2802. doi:10.1111/cas.14442, PMID:32357278.
[44] Laudadio I, Carissimi C, Fulci V. How RNAi machinery enters the world of telomerase. Cell Cycle 2019;18(10):1056–1067. doi:10.1080/15384101.2019.1609834, PMID:31014212.
[45] Laudadio I, Orso F, Azzalin G, Calabrò C, Berardinelli F, Coluzzi E, *et al*. AGO2 promotes telomerase activity and interaction between the telomerase components TERT and TERC. EMBO Rep 2019;20(2):e45969. doi:10.15252/embr.201845969, PMID:30591524.
[46] Diao C, Guo P, Yang W, Sun Y, Liao Y, Yan Y, *et al*. SPT6 recruits SND1 to co-activate human telomerase reverse transcriptase to promote colon cancer progression. Mol Oncol 2021;15(4):1180–1202. doi:10.1002/1878-0261.12878, PMID:33305480.