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# Circular RNA: An Important Biomolecule to Control Gastric Cancer

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***Abstract*—Recent studies with circRNAs indicate that circRNAs can act as important biomarkers for different lethal diseases, including cancer. The involvement of several RNAs, including circRNAs, with cancer is well established. Nevertheless, the underlying mechanism and regulation of many circRNAs is yet to be established. Our group has previously reported the unique link between circMAPK1 and circPGD, the circRNAs, which were found to be associated with gastric cancer. CircPGD was found to be the positive regulator, and circMAPK1 is the negative regulator of gastric cancer. Unlike circMAPK1 and circPGD, circDLG1 was also found to have progressive impacts on gastric cancer. Based on the evidence from the literature search, we identified these three circRNAs that have their abilities to control gastric cancer, and they exert their roles by sponging certain miRNAs. The preliminary information about those three circRNAs was obtained from the circBase and circBank databases. The information about the common miRNAs associated with those three circRNAs were obtained from miRDB database. To corroborate the previous findings, information from the DISEASE database was used. The objective of this study was to predict the mechanism of action of these circular RNAs and also to report the associated genes (if any), which are related to cancer, focusing on gastric cancer. miRNA (mir-141-3p) was found to play a significant role and have a sponging site on circDLG1. Prediction of the functional genes associated with the miRNAs and these three circRNAs was also investigated, and it was observed that they can influence numerous physiological processes and malignancies, including gastric cancer. In a nutshell, the study reports the association of the three important circRNAs with various cancer pathways, including gastric cancer, which might be useful in future drug discovery processes.***

***IndexTerms*—Gastric cancer; CircRNA; miRNA; Database search; Genes involved.**

## I. INTRODUCTION

Nowadays, research on non-coding RNAs is becoming more popular across a variety of disciplines. Although they are first thought of as transcriptional junks, their significance in several physiological processes makes them more significant in therapeutic aspects. This particular type of ncRNA in the RNA family known as circular RNAs, or circRNAs, are important players in a wide range of biological activities. These days, many researchers are interested in circRNAs because of their single-stranded, covalently closed, distinct structural arrangement, tissue-specific role, and participation in a number of developmental events [1-3]. They are derived from the "back splicing" of antecedent mRNAs [4]. According to their origin, they are classified into three categories: i) exonic, ii) intronic, and iii) exon-intronic cirRNA. CircRNAs are basically covalently closed-loop structures that have a lack of 5' capping and 3' polyadenylated tail [5]. As per their structural configuration, they are stable forms of RNA molecules and resistant to RNAseR degradation. Numerous studies revealed that circRNAs have also gained attention recently because of their critical functions in a number of metabolic processes through various mechanisms, such as iRES-mediated translation, RNA-binding protein (RBP) mechanisms, microRNA (miRNA) sponging, and others [6] (shown in Fig. 1.).

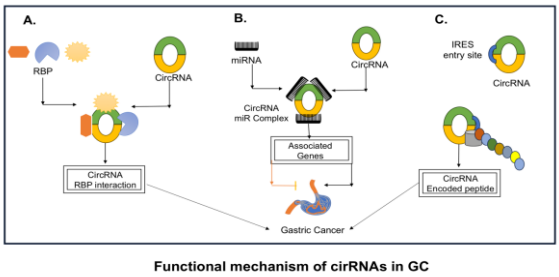


Fig. 1. There are several mechanisms by which circular RNA can work. A. RBPs bound with circRNAs, B. circRNAs sponged with miRNAs, and C. circRNAs started with IRES-mediated translation.

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Some of the circular RNAs have protein-coding potentials, and their resulting peptides/proteins are related to several biochemical processes and diseases, including cancer, neurological disorders, etc. Recent research has shown that specific circular RNAs independently contribute to the pathophysiological processes of cancer cells through protein post-transcriptional modification, cell cycle regulation, apoptosis, angiogenesis, metastasis, migration, and invasion [7]. Additionally, these circRNAs are thought to be possible biomarkers in certain situations and govern a number of disorders [8].

In this study, we looked at three different circRNAs that are linked to gastric cancer (GC). circMAPK1 (hsa_circ_0004872), circPGD (hsa_circ_0009735), and circDLG1 (hsa_circ_0008583) are the first three. Jiang et al. (2021) [9] claim that circMAPK1 has the ability to code for proteins and may interact competitively with MEK1 to block the MAP kinase pathway. However, according to Liu et al. (2022) [10] and Chen et al. (2021) [11], circPGD codes for a distinct oncogenic peptide that binds to the ABL2 gene axis, suppresses the miR-16-5p via the SMAD2/3 and YAP signalling pathways, inhibits apoptosis, and promotes cell proliferation. In contrast, circDLG1, which is derived from the DLG1 gene, directly interacts with the miR-141-3p, acting as a miRNA sponge to increase the expression of the miR-141-3p target gene chemokine 12 (CXCL12), thereby promoting the growth of GC. These three different circRNAs have demonstrated conflicting functions in GC. We aimed to identify the hallmarks of these opposingly active circular RNAs' effects on GC through their interactions with various miRNA sponging mechanisms. These three distinctive circular RNAs, the miRNAs they are associated with, and the pertinent genes are not established or connected in gastric cancer. This is the first attempt of its sort that might lead to the creation of new therapies in the future. So, the goal of this work is to determine which miRNA binding sites are shared by the three separate circRNAs circMAPK1, circPGD, and circDLG1. Next identification of the common gene pool associated with common miRNAs and their roles throughout the development of gastric cancer is shown in Fig. 2.

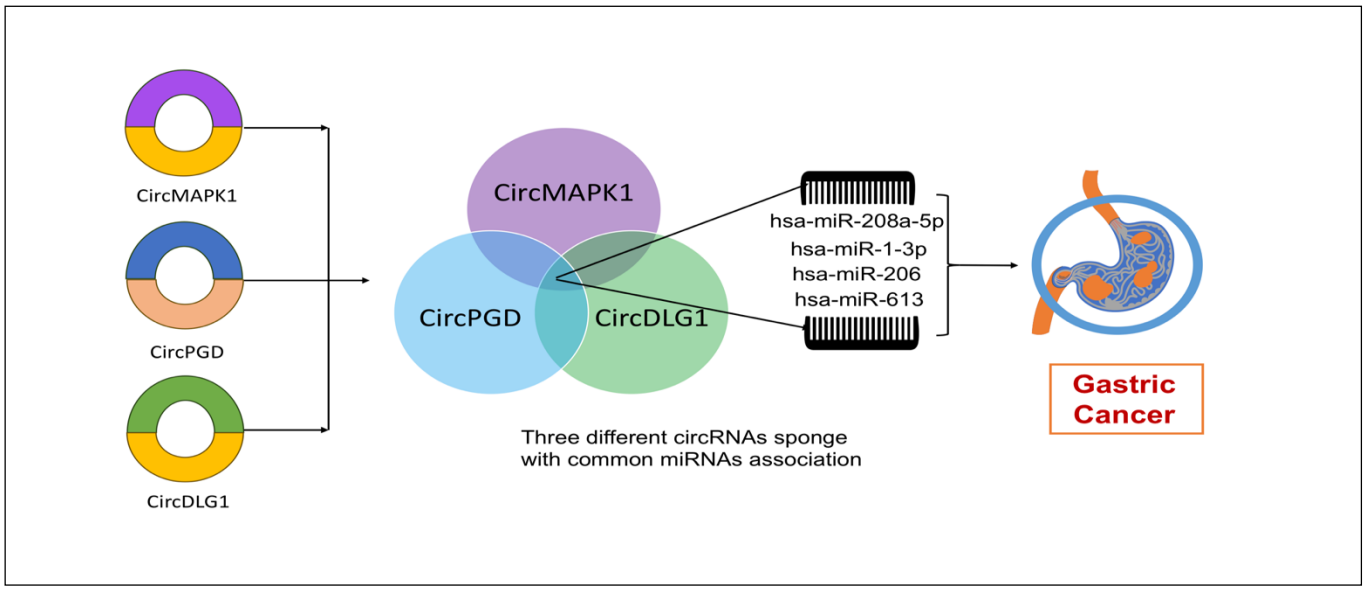


Fig. 2. Gastric cancer is regulated by three different circRNAs and their shared associated miRNAs.

## II. METHODS

### *A. Sequence Retrieval:*

In order to provide an illustration of the circRNAs, we acquired their respective IDs from the literature studies. Following that, we obtained this circRNA ID and fetched other information from different databases—circBase (http://www.circbase.org/) and another is circbank (http://www.circbank.cn/#/home). From these two databases, information on different miRNA binding sites was gathered, including the length of circular RNAs and complementary nucleotide sequences. In order to provide an illustration of the circRNAs, we acquired their respective IDs from the literature studies. The circRNAs' corresponding IDs from the literature research was obtained. Thereafter, the unique circIDs generated by the literature mining method were used to search the records at circBase and circBank to find the information that matched them. A variety of data was collected, such as circular RNA length, complementary nucleotide sequences, knowledge of various

miRNA binding sites, and knowledge of miRNA interactions. Also, the data was collected from the miRNA sponging experiment shortly after the sequence sorting was finished, and we used the Venn diagram tool to identify the common miRNAs that these three circRNAs share.

*B. Sorting of Data:*

In the next step the common miRNAs are then fetched in another database that is miRDB (https://mirdb.org/). This gives us information about the relevant genes linked to these miRNAs that are either GC-targeted or GC-connected. The Venn diagram tool was used to further assist in this process.

*C. Analysis of Data:*

The GC percentage of the selected circRNAs was calculated using the GC calculator tool. Following that, we gathered the miRNA-linked genes associated with the circular RNAs relevant to GC. Additionally, the entire GC data set from the DISEASE database (https://diseases.jensenlab.org/Search) was extracted, which is another database. These disease gene data sets are all supported by literature and experimental findings. In order to determine which gene sets are more frequent to GC onset, these two data sets were compared. These correlations helped to guide our further research on the relationships between circRNAs and GC.

## III. RESULTS AND DISCUSSION

We have found, circMAPK1, circPGD and circDLG1 with their unique circBase IDs, that is has_circ_0004872, has_circ_0009735, and hsa_circ_0008583. Utilizing the circBase and circBank databases, we were able to gather all of the available genomic data, such as chromosomal location, strand sense, genome length, and length of the spliced transcript. The GC content was determined for each spliced transcript sequence that we obtained from the circBank database. TABLE I displays the GC content percentages of these circRNAs, which are 44.5%, 51.6%, and 44.6%, respectively. The percentage of GC content signifies GC-rich sequences can serve as binding sites for RBPs and miRNAs. CircRNAs with specific GC content may have higher affinity for particular RBPs, which can modulate their function, localization, or stability and also enhance sponges for miRNAs. GC content may affect the binding efficiency and specificity of circRNAs to miRNAs, given the complementarity required for stable interactions. Furthermore, GC-rich sequences may enhance the efficiency of translation by stabilizing IRES structures. Higher percentages of GC content indicate the exonic form of circRNAs, which are more capable of coding proteins (Guha et al, 2024) [6]. We also collected the miRNA data from circBank to acquire a huge number of sponging miRNAs. Four common miRNAs (hsa-miR-208a-5p, hsa-miR-1-3p, hsa-miR-206, and hsa-miR-613) that are connected to every circRNA may be found using the Venn diagram tool. Although some of them affect tumor growth, chemoresistance, and the tumor microenvironment, miRNAs generally function primarily as tumor suppressors in a variety of malignancies. Hsa-miR-208a-5p possesses the tumor-progressive characteristic [12], whereas possesses-miR-1-3p, hsa-miR-206, and hsa-miR-613 exhibit the suppressive effect [13-15].
Additionally, these four miRNAs were sorted and their associated gene list filtered from the miRDB database that may act in various biochemical or disease pathways. After that, we analyzed the gene list and compared it with the gastric cancer gene data from the DISEASE database and used the Venn diagram tool to furtherdetect the commonalities in the two gene sets. This data is shown below the TABLE I.

TABLE I: Comprehensive data on the common miRNAs and circular RNAs implicated in the metabolic process, together with the GC percentage and state of regulation

| ***CircRNA*** | ***GC percentage*** | ***Common miRNA interaction*** | ***Associated Gene*** | ***Regulation status on GC*** | ***Reference*** |
|---|---|---|---|---|---|
| circMAPK1 (hsa_circ_0004872) | 44.5% | | | | |
| | | hsa-miR-208a-5p | | | |
| | | | CDK6 | UP | [16] |
| | | hsa-miR-1-3p | MET | UP | [17] |
| circPGD (hsa_circ_0009735) | 51.6% | | KRAS | UP | [18,19] |
| | | hsa-miR-206 | ASH1L | UP | [20] |

| | | | | | |
|---|---|---|---|---|---|
| | | | FBXW7 | DOWN | [21,22] |
| | | hsa-miR-613 | | | |
| circDLG1 (hsa_circ_0008583) | 44.6% | | | | |

Five genes are getting after each of these processes: CDK6, MET, KRAS, ASH1L, and FBXW7. These genes have both regressive and progressive roles in the development of gastric cancer, as seen in Fig. 3. and TABLEI. Of these five genes, FBXW7 is a negative regulatory gene that can downregulate gastric cancer[21], whereas CDK6, MET, KRAS, and ASH1L are positive regulatory genes that upregulate gastric cancer[16-20] Furthermore, this FBXW7 controls cell division, proliferation, and other processes and plays a major part in the MAPK pathways[22]. The two opposing groups of circular RNAs' functioning can be indicated by this.

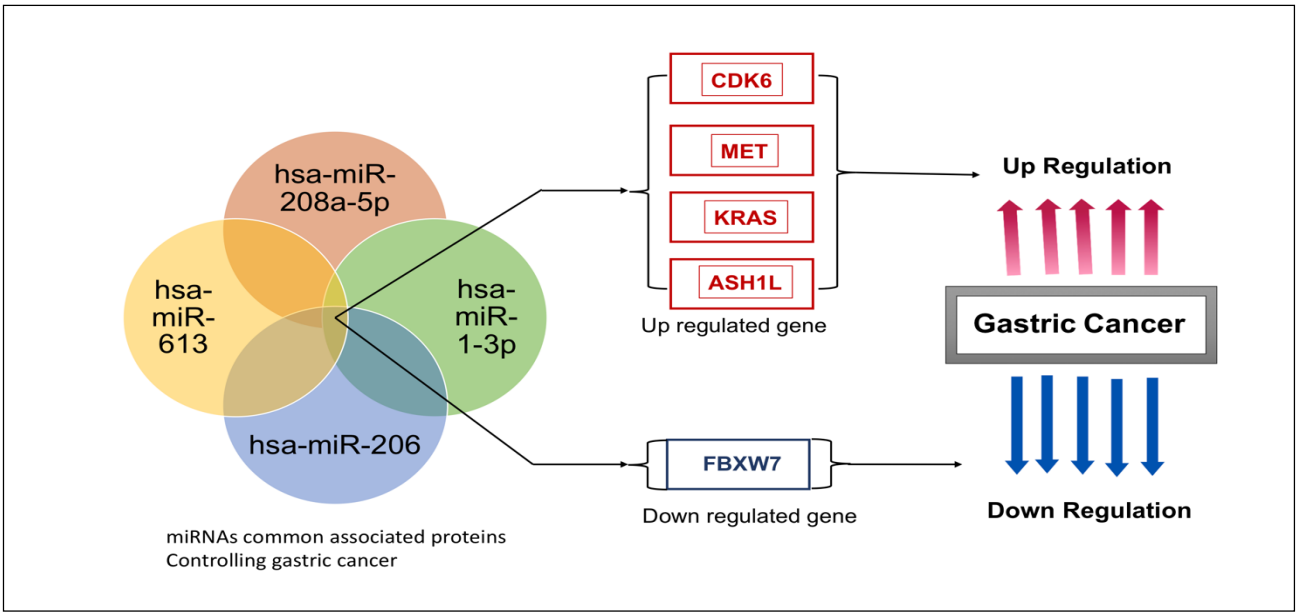


Fig. 3. Four miRNAs associated with common genes and GC disease common shared gene can up- or down-regulate the gastric cancer.

Circular RNAs are being investigated as a possible biomarker for the prognosis of gastric cancer. However, there hasn't yet been a study that compares different kinds of antagonistic circRNAs. Thus, our work is unique in that it demonstrates the discriminating properties of these RNAs.

## IV. CONCLUSION AND FUTURE PROSPECTS

This study allows us to pinpoint a few miRNA-sponging genes that are linked to three distinct antagonistic circRNAs. By controlling several genes that may be important regulators of the disease, these genes, which share a common gene pool with miRNAs, may have both beneficial and detrimental impacts on the regulation of gastric cancer.This work can concentrate on the role and connection of additional related and unrelated circRNAs in gastric cancer and other conditions.Therefore, our next goal is to characterize these circRNAs, their binding interactions with the miRNAs specific locations, and other genes and how they regulate gastric cancer.

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## CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

JM: Writing the original draft, Investigation, Formal analysis, Data curation.
DG: Review & Editing.
AB: Conceptualization, Validation, Project administration, Review & Editing.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT OF PUBLICATION

All the authors approved the final version of the manuscript for publication.

## AVAILIBILITY OF DATA AND MATERIAL

Data will be made available upon request.

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## REFERENCES

[1] Ebbesen KK, Hansen TB, Kjems J. Insights into circular RNA biology. RNA Biol. 2017 Aug 3;14(8):1035-1045. doi: 10.1080/15476286.2016.1271524. Epub 2016 Dec 16. PMID: 27982727; PMCID: PMC5680708.

[2] Qu S, Zhong Y, Shang R, Zhang X, Song W, Kjems J, Li H. The emerging landscape of circular RNA in life processes. RNA Biol. 2017 Aug 3;14(8):992-999. doi: 10.1080/15476286.2016.1220473. Epub 2016 Aug 11. PMID: 27617908; PMCID: PMC5680710.

[3] Salzman J. Circular RNA Expression: Its Potential Regulation and Function. Trends Genet. 2016 May;32(5):309-316. doi: 10.1016/j.tig.2016.03.002. Epub 2016 Apr 2. PMID: 27050930; PMCID: PMC4948998.

[4] Liu H, Hao W, Yang J, Zhang Y, Wang X, Zhang C. Emerging roles and potential clinical applications of translatable circular RNAs in cancer and other human diseases. Genes Dis. 2022 Oct 29;10(5):1994-2012. doi: 10.1016/j.gendis.2022.10.015. PMID: 37492726; PMCID: PMC10363642.

[5] Zhang J, Zhang X, Li C, Yue L, Ding N, Riordan T, Yang L, Li Y, Jen C, Lin S, Zhou D, Chen F. Circular RNA profiling provides insights into their subcellular distribution and molecular characteristics in HepG2 cells. RNA Biol. 2019 Feb;16(2):220-232. doi: 10.1080/15476286.2019.1565284. Epub 2019 Jan 18. PMID: 30614753; PMCID: PMC6380345.

[6] Guha, D., Mondal, J., Nandy, A., Biswas, S., & Bagchi, A. (2024). Interplay of circular RNAs in gastric cancer-a systematic review. *Frontiers in Systems Biology*, *4*, 1497510.

[7] Liu CX, Chen LL. Circular RNAs: Characterization, cellular roles, and applications. Cell. 2022 Jun 9;185(12):2016-2034. doi: 10.1016/j.cell.2022.04.021. Epub 2022 May 17. Erratum in: Cell. 2022 Jun 23;185(13):2390. doi: 10.1016/j.cell.2022.06.001. PMID: 35584701.

[8] Verduci, L., Tarcitano, E., Strano, S., Yarden, Y., & Blandino, G. (2021). CircRNAs: role in human diseases and potential use as biomarkers. *Cell death & disease*, *12*(5), 468.doi.org/10.1038/s41419-021-03743-3. PMID: 33976116; PMCID: PMC8113373.

[9] Jiang T, Xia Y, Lv J, Li B, Li Y, Wang S, Xuan Z, Xie L, Qiu S, He Z, Wang L, Xu Z. A novel protein encoded by circMAPK1 inhibits progression of gastric cancer by suppressing activation of MAPK signaling. Mol Cancer. 2021 Apr 9;20(1):66. doi: 10.1186/s12943-021-01358-y. PMID: 33836754; PMCID: PMC8034133.

[10] Liu Y, Cao J, Zhu L, Zhao W, Zhou Y, Shao C, Shao S. Circular RNA circPGD contributes to gastric cancer progression via the sponging miR-16-5p/ABL2 axis and encodes a novel PGD-219aa protein. Cell Death Discov. 2022 Sep 14;8(1):384. doi: 10.1038/s41420-022-01177-0. PMID: 36104322; PMCID: PMC9472197.

[11] Chen DL, Sheng H, Zhang DS, Jin Y, Zhao BT, Chen N, Song K, Xu RH. The circular RNA circDLG1 promotes gastric cancer progression and anti-PD-1 resistance through the regulation of CXCL12 by sponging miR-141-3p. Mol Cancer. 2021 Dec 15;20(1):166. doi: 10.1186/s12943-021-01475-8. PMID: 34911533; PMCID: PMC8672580.

[12] Barani A, Beikverdi K, Mashhadi B, Parsapour N, Rezaei M, Javid P, Azadeh M. Transcription Analysis of the *THBS2* Gene through Regulation by Potential Noncoding Diagnostic Biomarkers and Oncogenes of Gastric Cancer in the ECM-Receptor Interaction Signaling Pathway: Integrated System Biology and Experimental Investigation. Int J Genomics. 2023 Dec 22;2023:5583231. doi: 10.1155/2023/5583231. PMID: 38162289; PMCID: PMC10756743.

[13] Ma J, Zhu M, Ye X, Wu B, Wang T, Ma M, Li T, Zhang N. Prognostic microRNAs associated with phosphoserine aminotransferase 1 in gastric cancer as markers of bone metastasis. Front Genet. 2022 Aug 19;13:959684. doi: 10.3389/fgene.2022.959684. PMID: 36061202; PMCID: PMC9437321.
[14] Yang Q, Zhang C, Huang B, Li H, Zhang R, Huang Y, Wang J. Downregulation of microRNA-206 is a potent prognostic marker for patients with gastric cancer. Eur J Gastroenterol Hepatol. 2013 Aug;25(8):953-7. doi: 10.1097/MEG.0b013e32835ed691. PMID: 23751352.
[15] Raei N, Yazdanbod A, Latifi-Navid S, Safaralizadeh R. Diagnostic value of long noncoding RNA SNHG15 in gastric cancer: *in vitro* and *in silico* studies. Nucleosides Nucleotides Nucleic Acids. 2024;43(12):1459-1471. doi:10.1080/15257770.2024.2320224. Epub 2024 Feb 22. PMID: 38386862.
[16] Zhang J, Chen B, Wang Y, Liu X, Yan H, Wong KY, Chan AK, Cheung AH, Chow C, Xu D, Wang S, Huang B, Liang L, Ke H, Wong CC, Wu WK, Cheng AS, Yu J, Lo KW, To KF, Kang W. The E2F1-HOXB9/PBX2-CDK6 axis drives gastric tumorigenesis and serves as a therapeutic target in gastric cancer. J Pathol. 2023 Aug;260(4):402-416. doi: 10.1002/path.6091. Epub 2023 Jun 5. PMID: 37272544.
[17] Hyung S, Ko J, Heo YJ, Blum SM, Kim ST, Park SH, Park JO, Kang WK, Lim HY, Klempner SJ, Lee J. Patient-derived exosomes facilitate therapeutic targeting of oncogenic MET in advanced gastric cancer. Sci Adv. 2023 Nov 24;9(47):eadk1098. doi: 10.1126/sciadv.adk1098. Epub 2023 Nov 24. PMID: 38000030; PMCID: PMC10672184.
[18] Liu J, Ji S, Liang C, Qin Y, Jin K, Liang D, Xu W, Shi S, Zhang B, Liu L, Liu C, Xu J, Ni Q, Yu X. Critical role of oncogenic KRAS in pancreatic cancer (Review). Mol Med Rep. 2016 Jun;13(6):4943-9. doi: 10.3892/mmr.2016.5196. Epub 2016 Apr 27. PMID: 27121414.
[19] Won Y, Choi E. Mouse models of Kras activation in gastric cancer. Exp Mol Med. 2022 Nov;54(11):1793-1798. doi: 10.1038/s12276-022-00882-1. Epub 2022 Nov 11. PMID: 36369466; PMCID: PMC9723172.
[20] Xie M, Zhang L, Han L, Huang L, Huang Y, Yang M, Zhang N. The ASH1L-AS1-ASH1L axis controls NME1-mediated activation of the RAS signaling in gastric cancer. Oncogene. 2023 Nov;42(46):3435-3445. doi: 10.1038/s41388-023-02855-8. Epub 2023 Oct 7. PMID: 37805663.
[21] Sun J, Bai YK, Fan ZG. Role of FBXW7 expression in gastric cancer: Meta-analysis and bioinformatics analysis. Oncol Lett. 2023 Mar 24;25(5):184. doi: 10.3892/ol.2023.13770. PMID: 37113395; PMCID: PMC10126736.
[22] Pan Y, Liu J, Gao Y, Guo Y, Wang C, Liang Z, Wu M, Qian Y, Li Y, Shen J, Lu C, Ma S. FBXW7 loss of function promotes esophageal squamous cell carcinoma progression via elevating MAP4 and ERK phosphorylation. J Exp Clin Cancer Res. 2023 Mar 29;42(1):75. doi: 10.1186/s13046-023-02630-3. PMID: 36991467; PMCID: PMC10054043.