HUMAN BIOLOGY
2024, VOL. 95, ISSUE 1
ORIGINAL ARTICLE

OPEN ACCESS

Ribosome Biogenesis: A Major Player in The Exosome of Gastric Cancer

Chun-yan Lv[1], Xian-kui Cheng[2], Yuan Zhong[1], You-li jian[3], Miming He[1], Tao Lei[1]*

ABSTRACT

Background: To identify of vital genes in the blood exosome related to the process of gastric carcinogenesis and help to reduce mortality rates through early diagnosis and the development of new anticancer therapies.

Methods: The RNAs data of blood exosomes from patients with GC and healthy controls were downlinked from exoRBase database, and the differential expression of messenger RNA (mRNA), long non coding RNA (lncRNA), and circular RNA (circRNA) were analyzed using R language. Then the relevant RNAs and their corresponding miRNA data predicted by ENCORI, miRcode and other databases, were imported into the ceRNA network. Finally, the DAVID was accessed to investigate the DEmRNAs' GO annotations and KEGG analysis.

Results: A total of 62 mRNAs, 3 lncRNAs and 15 circRNAs were differentially expressed. The ceRNA network was constructed with Cytoscape software, including 192 mRNA nodes, 32 lncRNA nodes, 28 circRNA nodes, and 152 miRNA nodes, with the top 10 hub genes EMSY, ZEB2, LCOR, MFSD14A, ERBIN, hsa-miR-363-3p, hsa-miR-137, hsa-miR-27a-3p, hsa-miR-23b-3p and hsa_circ_0000038. According to GO annotation, the biological processes mainly in cytoplasmic translation, the cell component were mostly in the ribosome and its subunit and the major molecular functions included structural constituent of ribosome, etc. The KEGG revealed that the DEmRNA were primarily enriched in mRNA monitoring pathway.

Conclusion: A ceRNA network in the blood exosome of GC were built, the hub genes were screened out, the biological process, cell component, molecular function and enrichment pathway of DEmRNA were explored, which proved that the ribosome biogenesis was a major player in the exosome of gastric cancer and this provide precise targets for making a diagnosis and giving treatment of GC.

INTRODUCTION

Gastric cancer (GC) is a kind of cancer that occurs in the stomach, mostly from gastric mucosal epithelial cells. The most common pathological type is adenocarcinoma. The long-term survival rate (>5 years) of early GC after surgery can reach 90.9%~100% The Information Committee of the Korean Gastric Cancer Association The Information Committee of the Korean Gastric Cancer Association. (2021), Hamashima.(2018), Park et al.(2022). However, there is still no effective treatment for advanced GC. Even with various treatments, the survival rate of 5-year is still less than 30%. The incidence rate of GC ranks the fifth in malignant tumors and the third in mortality worldwide. Early detection of early GC or postoperative recurrence of GC is still the focus of cancer prevention and treatment Chen et al.(2023). The exosome is extracellular vesicle with the diameter of 30-150 nm, whice is released into the extracellular matrix after the outer membrane fuses with the cell membrane Khazaei et al.(2023). They have lipid bilayer and contain mRNA, lncRNA, circRNA, and transfer RNA (tRNA), etc. Rangel-Ramírez et al.(2023), which are vital for tumor cell biology, such as proliferation Richards et al.(2017) and metastasis Huang et al.(2022), Nambara et al.(2023), also can serve as prognostic markers and/or grading basis for patients with tumors, belong to the research field of liquid biopsy Zhang et al.(2022), Chaudhry et al.(2022), Rzhevskiy et al.(2022).

In this study, we obtained the RNA (including mRNA, lncRNA and circRNA) information of plasma exosome samples from patients with GC and healthy people from exoRBase database Zhu et al.(2022), Wu et al.(2022) ,Lai et al.(2022), screened the differentially exosomal RNAs in GC using bioinformatics methods,

[1] School of Basic Medicine, Chengdu University, Chengdu, China,
[2] Department of Pathology, Provincial Hospital Affiliated to Shandong Medical University, Jinan, China,
[3] Department of Pathology, Pidu District People's Hospital, Chengdu, China

Correspondence to: Chunyan-Lv, Basic Medical College ,Chengdu University, Chengdu 610106,P.R. China.
E-mail : Chuanyan-l@foxmai.com,Tel: +8615908118468.

Keywords: gastric cancer; exosome; enrichment analysis; ribosome biogenesis

constructed a network related to GC with the competitive endogenous RNA (ceRNA). The functional enrichment analysis of mRNA is also carried out in order to discover more new GC markers and provide guidance for early detection, early identification and early therapy of GC.

MATERIALS AND METHODS

Data source

The expression profiles of RNAin gastric cancer(GC) and healthy blood samples were download from exoRBase2.0. The data deadline was December 27, 2022. The data format was pretreated. 9 blood samples from GC patients and 118 healthy blood samples were obtained, involving mRNA and lncRNA35517 and circRNAs 79084.

Filtering of diversely emanated genes (DEGs)

The R language "limma" package was applied to take the mean value and the "sva" package was employed for batch correction to identify the DEGs. The filtering condition for DEGs was P-value < 0.05. The corresponding volcano map was drawn with the "heat map" package of top 20 genes with obvious differences.

Prediction of miRNAs and Construction of ceRNA Networks

Target Scan Human and miRanda in ENCORI (https://starbase.sysu.edu.cn/) were employed to combinedly forecast the differentially express mRNAs-bound miRNAs. The miRNA Target function in ENCORI was applyed to forecast the miRNA binding to DEcircRNAs and the lncRNAs binding miRNAs were predicted in miRcode (http://www.mircode.org/). Finally, the prediction data of miRNAs and the corresponding mRNAs, circRNAs and lncRNAs were input into the Cytoscape software(version 3.7.2) to display graphically the ceRNA network.

Hub gene sifting

The cytoHubba plugin of Cytoscape software was utilized to pick the top 10 nodes out as Hub gene by the betweenness algorithm, and the topology index of the betweenness of each Hub node was used to plot in EXCEL.

Functional enrichment unpacking

The DEmRNAs were input to DAVID database(https://david.ncifcrf.gov/), with the identifier as "OFHCIAL-GENE-SYMBOL" and the species as "Homo sapiens", GO annotations and KEGG pathway analysis were carried according to the conditions of P<0.05 and FDR<0.05(FDR is the P value after correction and calculation).

Statistical methods

All statistical analyses were completed in R4.2.2 statistical software. The mean ± standard deviation was employed to display the measurement data, and t-test or analysis of variance was utilized to perform the statistical test. $P<0.05$ means the difference was statistically significant.

RESULTS

Results of DEGs screening

62 DemRNAs and 3 differentially expressed lncRNAs2 (all up-regulated genes) were screened out, with HNRNPK as the most significant mRNA, AC011450.1 exhibiting the most relevant lncRNA. The DEGs heat map was seen in Figure 1.

Figure 1: Heat map of mRNAs and lncRNAs in plasma exosomes of patients with GC and healthy(N)

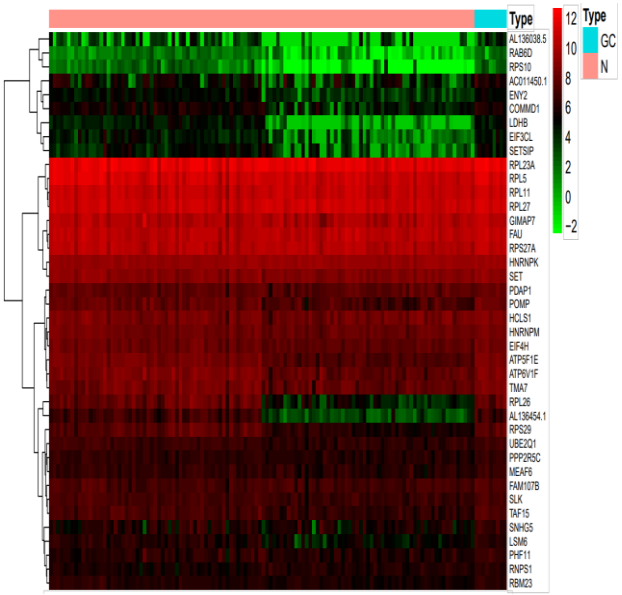
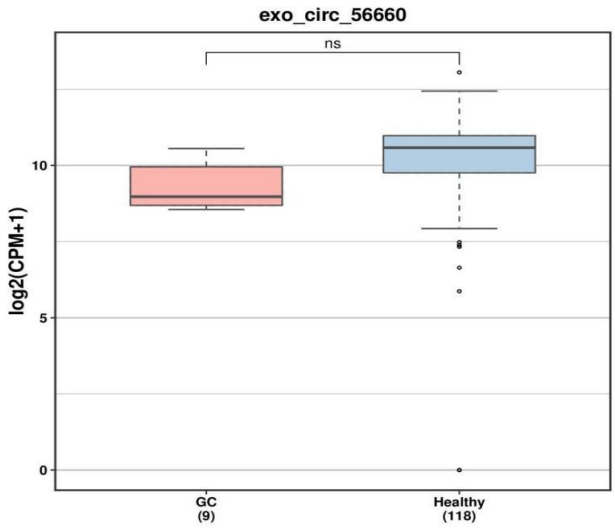


Figure 2: hsa_circ_0007476(exo_circ_56660) in plasma exosomes of patients with GC and healthy



One down regulated circRNAs (hsa_circ_0007476, Genomic position: chr4:53414615-53428183, Genomic length: 13568, Spliced length: 356) with differential expression (DECircRNAs) was shown in Figure2.

Establish of exosomal ceRNA network

According to the data of DEmRNAs, DElncRNAs, DEcircRNAs and their binding miRNAs, a competitiveness endogenous RNA network was constructed(Figure 3), with 192 mRNA nodes (involving 22 mRNA), 32 lncRNA nodes (1 lncRNAinvolved), 28 circRNA nodes (involving 1 circRNA) and 151 miRNA nodes. The top 10 pivot genes with the highest betweenness scores were distinguished by Cytoscape software, and were shown in Figure 4, including 5 mRNAs, 4 miRNAs and 1 circRNA.

Figure 3: The competitiveness endogenous RNA regulatory network related to miRNA

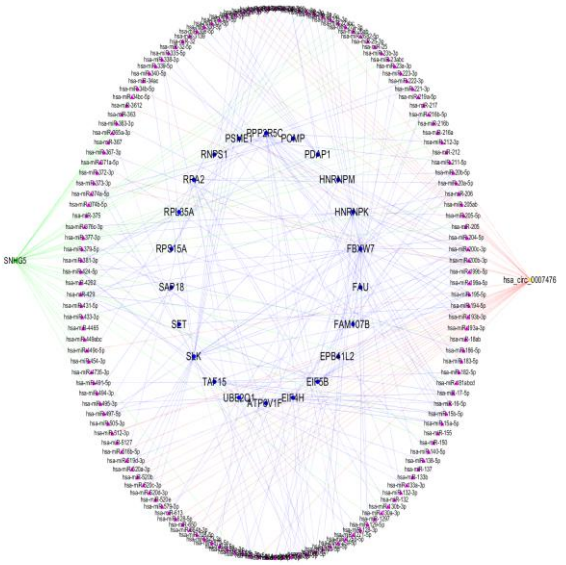
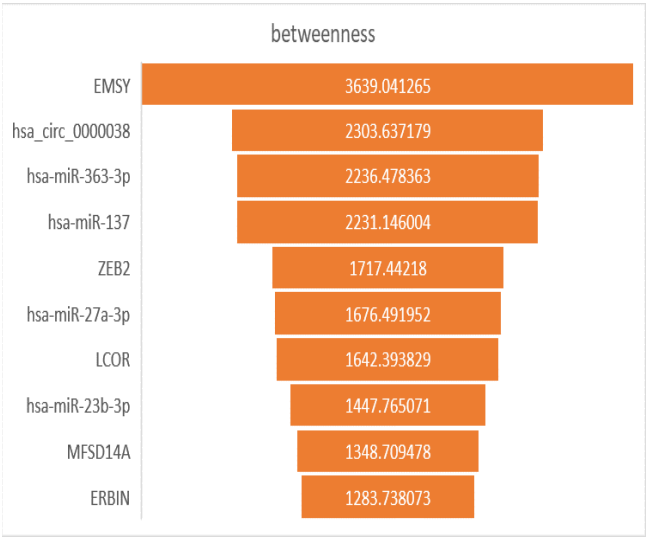


Figure 4: The first 10 pivot genes with the highest betweenness scores in exosomal ceRNAs

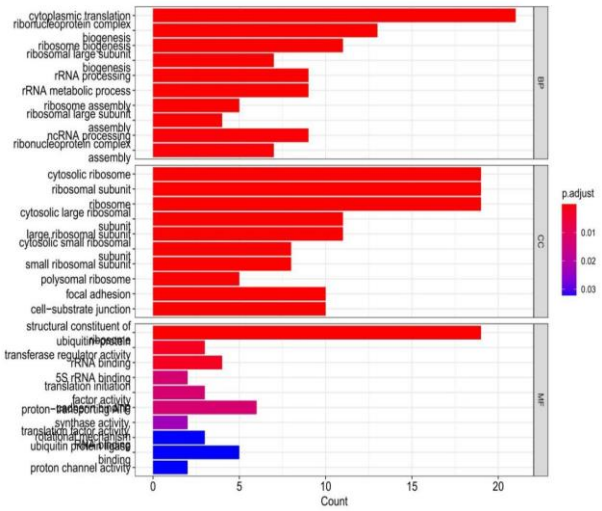


GO Annotation Analysis

The Gene Ontology of overlapping genes was shown in Figure 5. The biological processes of differential exosomal mRNA included cytoplasmic translation, ribosome (nucleoprotein complex, large subunit), rRNA processing, rRNA metabolic process, ribosome (large subunit, ribonucleoprotein complex) assembly and ncRNA processing. As we know, the occurrence of intestinal gastric cancer goes through a multi-step process, involving mutations such as P53 gene Yang et al.(2023), Yao et al.(2002), Luinetti et al.(1998), while diffuse gastric cancer involves the reduction or deletion of cadherin Jinawath et al.(2004), Rogers et al.(2008),

Figure 5: The bar chart (A) and bubble plot (B) of GO annotations

A



B

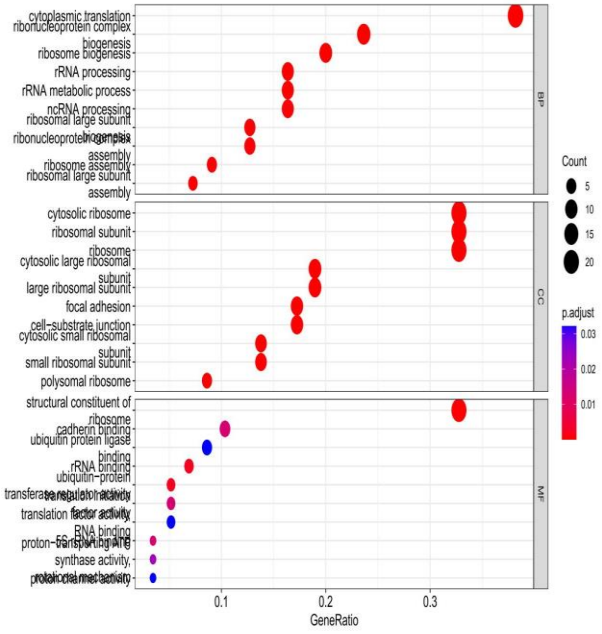


Table 1: The paths involved in the P53 gene

Ontology	Description	p-value	geneID	Count
BP	regulation of intrinsic apoptotic signaling pathway by p53 class mediator	0.000119539	HNRNPK/RPL11/RPL26	3
BP	regulation of signal transduction by p53 class mediator	0.000246564	HNRNPK/RPL11/RPL26/RPL5	4
BP	regulation of intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	0.001099219	HNRNPK/RPL26	2
BP	signal transduction by p53 class mediator	0.001606558	HNRNPK/RPL11/RPL26/RPL5	4
BP	intrinsic apoptotic signaling pathway by p53 class mediator	0.001693755	HNRNPK/RPL11/RPL26	3
BP	positive regulation of signal transduction by p53 class mediator	0.003431736	RPL11/RPL26	2

which is also detected in the biological processes involved in these differential genes, but not on the top 10 range. The paths involved in the former are exhibited in Table 1. The cell components where the genes located were mainly in the ribosome, its subunit(large and small subunit) and polyribosomes formed by ribosomes, focal adhesion, cell-substrate junction, proton-transporting ATPase (two-sector) and ATP synthase complex(catalytic domain), endoplasmic reticulum membrane(its cytoplasmic side, rough endoplasmic reticulum and its membrane). Molecular functions included protein translation and modification, cadherin binding, rotational mechanism and proton channel activity, etc.

Enrichment analysis of KEGG

The KEGG pathway shows that the DEmRNA is mainly concentrated in the mRNA monitoring pathway (involving mRNA PPP2R5C, RNPS1, SAP18), proteasome (involving mRNA POMP and PSME1), ribosome (assuming mRNA FAU, RPL35A and RPS15A), as shown in Figure 6.

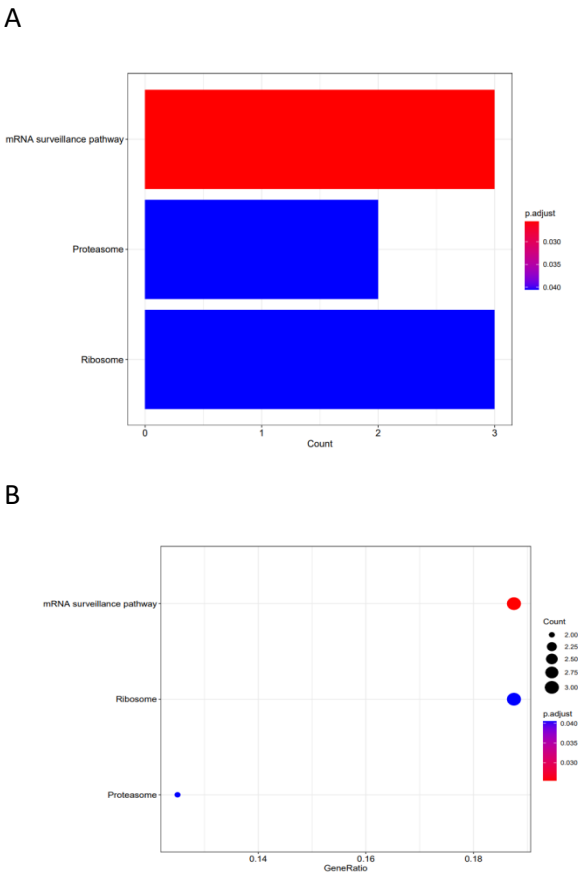


Figure 6: Column Chart (A) and Bubble map (B) of KEGG Analysis

DISCUSSIONS

Gastric cancer (GC) is one of most often malignancy of the alimentary system. The mortality of GC is high and the prognosis is unsatisfactory because of the detection often in the late stage of diagnosis Venkatasamy et al.(2023). Therefore, it is crucial to reduce the incidence rate and mortality of GC by early detection and the use of effective screening methods Jiang et al.(2022), Hengmin et al.(2022).

Exosomes are small vesicles excreted by living cells, with typical lipid bilayer membrane, carrying a variety of meaningful message such as proteins, lipids, nucleic acids, etc., play an significant role in the transmission of intercellular substances and information, and gradually become a biomarker for early diagnosis of many diseases Raez et al.(2023), Fujii et al.(2023), Zhang et al.(2023). Nucleic acids include DNA and RNA (mRNA, miRNA, lncRNA and tRNA), which provides a good data source for us to establish an competitive endogenous regulatory network of multiple RNAs Salmena et al.(2011), Smillie et al.(2018).

In this study, 62 DEmRNA, 3 DElncRNAs, and 1 DEcircRNAs were searched out, and the ceRNA system was built with their binding miRNA prediction data. Then, first 10 pivot genes with the highest betweenness scores were distinguished by Cytoscape software including 5 mRNAs(EMSY, ZEB2, LCOR, MFSD14A and ERBIN), 4 miRNAs(hsa-miR-363-3p, -137, -27a-3p and -23b-3p) and 1 circRNA (hsa_circ_0000038). The GO annotation and the enrichment analysis of KEGG pathway showed that ribosome biogenesis played a major role in the exosome of gastric cancer.

It is reported that ZEB2 expression was be relevant to the efficacy of platinum chemotherapy and the miR-338-5p-ZEB2 axis has diagnostic and prognostic value Wei et al.(2021). ZEB2, as an epithelial mesenchymal transformation regulator, inhibited by miR-200c, could inhibit the metastasis of non small cell lung cancer Jiao et al.(2021). The highly expressed lncRNA ZEB2-AS1 in GC patients had a poor prognosis Wang et al.(2019).The high expression of LCoR was considered as a poor prognosis marker in GC You et al.(2022). ERBB2 interacting protein (ERBB2IP) was up-regulated gene, which might be in connection with the resistance to i.p. paclitaxel in GC with peritoneal metastasis Huang et al.(2021). Hsa-miR-363-3p was probably a therapeutic targets and down-regulated in Tibetan with GC Luo et al.(2015). MiR-137 was negative correlation with EZH2 and its expression was down-regulated in GC Weng et al.(2022). Hsa-miR-27a-3p might serve critical roles in early GC Liang et al.(2021). However, the role of EMSY, MFSD14A, hsa-miR-23b-3p and hsa_circ_0000038 in the development and diagnosis of GC were still unclear, which could be used as potential targets for future research.

CONCLUSIONS

To sum up, this study used bioinformatics methods to identify exosomal DeRNA (mRNA, lncRNA and circRNA) related to GC occurrence, constructed corresponding ceRNA networks, and speculate that ribosome biogenesis played a major role in the exosome of gastric cancer, which provided a molecular basis for further research on GC progress and metastasis, and provided new liquid biopsy biomarkers for early diagnosis of GC. However, due to the small sample size of disease exosomes, further clinical multicenter validation is required. Different types of gastric cancer, different pathogenesis of adherent cancer and nonadhesive cancer, and their differences in ceRNA also need to be clarified.

DECLARATIONS

Ethics approval and consent to participate

Redundant.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of materials and data

The datasets generated during the current study are availabe in the exoRBase2.0 repository[http://www.exorbase.org/], and the R language package are available in the [bioconductor] repository[http://www.bioconductor.org/install/].
The other primary devotions emerged in the research are contained in the text/ Complementary Data. Further requirements can contact with the corresponding author.

Competing interests

This research was conducted in the absence of any commercial or financial relationships and had no potential conflict of interest.

Funding

This study was sustained by the Department of Science and Technology Key R&D projects in Sichuan Provincial (grant number: 2022YFS0200).

Author contributions

Ming-ming He and Yuan Zhong: Data collection, Data analysis, Manuscript writing. Tao Lei, You-li Jian and Xian-kui Cheng: Data analysis. Chun-yan Lv: Project development and Critical revision of manuscript.

Acknowledgements

At the point of finishing this paper, I'd like to express my sincere thanks to all those who have lent me hands in the course of my writing this paper. I'd like to thank those leaders, teachers and working staff especially those in Chengdu University. Without their help, it would be much harder for me to finish my study and this paper.

REFERENCES

1.The Information Committee of the Korean Gastric Cancer Association. 2021 Sep. Korean gastric cancer association-led nationwide survey on surgically treated gastric cancers in 2019. J Gastric Cancer. 21(3):221-235.

2.Hamashima C. 2018 Jul 1.Update version of the japanese guidelines for gastric cancer screening. Jpn J Clin Oncol . 48(7):673-683.

3.Park SH, Kang MJ, Yun EH, et al. 2022 Jul.Epidemiology of gastric cancer in korea: Trends in incidence and survival based on korea central cancer registry data (1999–2019). J Gastric Cancer. 22(3):160-168.

4.Chen M, Chen K, Hou H, et al. 2023 May 1.Incidence and mortality trends in gastric cancer in the United States, 1992-2019. Int J Cancer. 152(9):1827-1836.

5.Khazaei F, Rezakhani L, Alizadeh M, et al. 2023 Feb. Exosomes and exosome-loaded scaffolds: Characterization and application in modern regenerative medicine. Tissue Cell .80:102007.

6.Rangel-Ramírez VV, González-Sánchez HM, Lucio-García C. 2023 Feb.Exosomes: from biology to immunotherapy in infectious diseases. Infect Dis (Lond). 55(2):79-107.

7.Richards K E, Zeleniak A E, Fishel M L, et al. 2017 Mar 30. Cancer -associated Fibroblast Exosomes Regulate Survival and Proliferation of Pancreatic Cancer Cells. Oncogene. 36(13):1770-1778.

8.Huang L, Wang F, Wang X, et al. 2022 Dec 23. M2-like macrophage-derived exosomes facilitate metastasis in non-small-cell lung cancer by delivering integrin αVβ3. MedComm (2020). 4(1):e191.

9.Nambara S, Masuda T, Hirose K, et al. 2023 Jan-Feb.Rab27b, a Regulator of Exosome Secretion, Is Associated With Peritoneal Metastases in Gastric Cancer. Cancer Genomics Proteomics. 20(1):30-39.

10.Zhang Z, Wu H, Chong W, et al. 2022 Oct 27. Liquid biopsy in gastric cancer: predictive and prognostic biomarkers. Cell Death Dis. 13(10):903.

11.Chaudhry GE, Akim AM, Safdar N, et al. 2022 Oct-Dec. Cancer and Disease Diagnosis - Biosensor as Potential Diagnostic Tool for Biomarker Detection. J Adv Pharm Technol Res. 13(4):243-247.

12.Rzhevskiy AS, Kapitannikova AY, Butnaru DV, et al. 2022 Dec 2.Liquid Biopsy in Diagnosis and Prognosis of Non-Metastatic Prostate Cancer. Biomedicines. 10(12):3115.

13.Zhu K, Wang Q, Wang L. 2022 Jul 20.Analysis of Competitive Endogenous RNA Regulatory Network of Exosomal Breast Cancer Based on exoRBase. Evol Bioinform Online. 18:11769343221113286.

14.Wu ZH, Li C, Zhang YJ, et al. 2022 Apr 27.Bioinformatics Study Revealed Significance of Exosome Transcriptome in Hepatocellular Carcinoma Diagnosis. Front Cell Dev Biol. 10:813701.

15.Lai H, Li Y, Zhang H, et al. 2022 Jan 7. exoRBase 2.0: an atlas of mRNA, lncRNA and circRNA in extracellular vesicles from human biofluids. Nucleic Acids Res. 50(D1):D118-D128.

16.Yang D, Shi M, You Q, et al. 2023 Aug. Tumor- and metastasis-promoting roles of miR-488 inhibition via HULC enhancement and EZH2-mediated p53 repression in gastric cancer. Cell Biol Toxicol. 39(4):1341-1358.

17.Yao T, Kajiwara M, Kuroiwa S, et al. 2002 Oct. Malignant transformation of gastric hyperplastic polyps: alteration of phenotypes, proliferative activity, and p53 expression. Hum Pathol. 33(10):1016-22.

18.Luinetti O, Fiocca R, Villani L, et al. 1998 Jul.Genetic pattern, histological structure, and cellular phenotype in early and advanced gastric cancers: evidence for structure-related genetic subsets and for loss of glandular structure during progression of some tumors. Hum Pathol. 29(7):702-9.

19.Jinawath N, Furukawa Y, Hasegawa S, et al. 2004 Sep 2. Comparison of gene-expression profiles between diffuse- and intestinal-type gastric cancers using a genome-wide cDNA microarray. Oncogene. 23(40):6830-44.

20.Rogers WM, Dobo E, Norton JA, et al. 2008 Jun.Risk-reducing total gastrectomy for germline mutations in E-cadherin (CDH1): pathologic findings with clinical implications. Am J Surg Pathol. 32(6):799-809.

21.Venkatasamy A, Guerin E, Reichardt W, et al. 2023 Mar. Morpho-functional analysis of patient-derived xenografts reveals differential impact of gastric cancer and chemotherapy on the tumor ecosystem, affecting immune check point, metabolism, and sarcopenia. Gastric Cancer. 26(2):220-233.

22.Jiang T, Mei L, Yang X, et al. 2022 Oct 4. Biomarkers of gastric cancer: current advancement. Heliyon. 8(10):e10899.

23.Hengmin Xu, Wenqing Li. 2022 Dec 12.Early detection of gastric cancer in China: progress and opportunities. Cancer Biol Med . 19(12):1622-1628.

24.Raez LE, Brice K, Dumais K, et al. 2023 Mar. Liquid Biopsy Versus Tissue Biopsy to Determine Front Line Therapy in Metastatic Non-Small Cell Lung Cancer (NSCLC). Clin Lung Cancer. 24(2):120-129.

25.Fujii N, Yashiro M, Hatano T, et al. 2023 Jan .CD9-positive Exosomes Derived from Cancer-associated Fibroblasts Might Inhibit the Proliferation of Malignant Melanoma Cells. Anticancer Res. 43(1):25-33.

26.Zhang X, Hou H, Jiang M, et al. 2023 Mar. Aberrant circulating tumor DNA methylation and exosomal microRNA biomarkers for early detection of colorectal cancer. Mol Biol Rep. 50(3):2743-2750.

27.Salmena L, Poliseno L, Tay Y, et al. 2011 Aug 5.A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 146(3):353-8.

28.Smillie CL, Sirey T, Ponting CP. 2018 Jun.Complexity of post-transcriptional regulation and modeling of ceRNA crosstalk. Crit Rev Biochem Mol Biol. 53(3):231-245.

29.Wei X, Zhu J, Zhang Y, et al. 2021 Sep 23. miR-338-5p-ZEB2 axis in Diagnostic, Therapeutic Predictive and Prognostic Value of Gastric Cancer. J Cancer. 12(22):6756-6772.

30.Jiao A, Sui M, Zhang L, et al. 2021 Aug. MicroRNA-200c inhibits the metastasis of non-small cell lung cancer cells by targeting ZEB2, an epithelial-mesenchymal transition regulator. Mol Med Rep. 24(2):573.

31.Wang F, Zhu W, Yang R, et al. 2019 Jun. LncRNA ZEB2-AS1 contributes to the tumorigenesis of gastric cancer via activating the Wnt/β-catenin pathway. Mol Cell Biochem. 456(1-2):73-83.

32.You W, Ouyang J, Cai Z, et al. 2022 Jul 7.Comprehensive Analyses of Immune Subtypes of Stomach Adenocarcinoma for mRNA Vaccination. Front Immunol. 13:827506.

33.Huang KK, Huang J, Wu JKL, et al. 2021 Jan 22. Long-read transcriptome sequencing reveals abundant promoter diversity in distinct molecular subtypes of gastric cancer. Genome Biol. 22(1):44.

34.Luo Y, Zhang C, Tang F, et al. 2015 Dec 12. Bioinformatics identification of potentially involved microRNAs in Tibetan with gastric cancer based on microRNA profiling. Cancer Cell Int. 15:115.

35.Weng XQ, Wang W. 2022.miR-137 Modulates Human Gastric Cancer Cell Proliferation, Apoptosis, and Migration by Targeting EZH2. Crit Rev Eukaryot Gene Expr. 32(4):31-40.

36.Liang Y, Zhao Y, Li L, et al. 2021 Aug. MicroRNA profiles in five pairs of early gastric cancer tissues and adjacent non-cancerous tissues. Oncol Lett. 22(2):595.