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The Application Value of Tumor-Educated Platelet RNA in Hepatocellular Carcinoma Patients

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Abstract: Hepatocellular carcinoma (HCC), a highly prevalent malignant tumor worldwide, exhibits insidious onset and rapid progression. Patients are often diagnosed at an advanced stage, missing the optimal treatment window. In liquid biopsy, Tumor-Educated Platelets (TEPs) reveal that tumors can alter platelet RNA expression profiles through multiple mechanisms, positioning them as novel sources of biomarkers for tumor diagnosis. This review systematically elucidates the biological characteristics of TEPs in HCC development and their RNA expression profiles, summarizing the diagnostic value of TEPs RNA in early HCC screening, pathological classification, and treatment monitoring, thereby providing theoretical reference for non-invasive HCC diagnosis.

Keywords: Hepatocellular Carcinoma; Tumor-educated Platelets; Liquid Biopsy; Diagnosis; RNA.

1. Introduction

Global cancer statistics from 2022 reveal a grim outlook for liver cancer prevention and control, with its incidence and mortality ranking sixth and third among all malignancies worldwide [1]. Hepatocellular carcinoma (HCC) constitutes about 85% of liver cancer cases [2]. HCC develops insidiously and progresses rapidly, with patient survival rates closely tied to disease stage: median survival exceeds 5 years in very early/early-stage patients; shortens significantly to approximately 2.5 years in intermediate-stage patients; further declines to about 2 years in advanced-stage patients; and reaches only about 3 months in end-stage patients [3]. Unfortunately, due to the nonspecific clinical manifestations of early-stage HCC, most patients are diagnosed at an intermediate or advanced stage [4].

Among current non-invasive imaging modalities, ultrasound demonstrates less than 50% sensitivity for detecting early-stage HCC[5] . Its diagnostic accuracy is significantly operator-dependent and influenced by patient characteristics (e.g., obesity), with limited ability to identify microlesions (<1 cm diameter) and poor performance in distinguishing benign from malignant lesions[6] . CT and MRI achieve overall diagnostic sensitivities of 72% and 65% for HCC, respectively. However, their sensitivity drops sharply for lesions <2 cm in diameter (CT: 48%;MRI: 62%), and detecting lesions <1 cm is even more challenging[4, 7] . Although MRI has approximately 90% specificity, its sensitivity is only about 60%, and AFP levels remain within the normal range in approximately 15%-30% of advanced patients [4, 8]. Although biopsy remains the current gold standard for HCC diagnosis, its false-negative rate can reach up to 30%, with actual clinical sampling rates below 15% [9]. It also carries potential risks of complications such as bleeding and needle-track metastasis. In summary, existing diagnostic technologies exhibit significant limitations in both sensitivity and specificity.

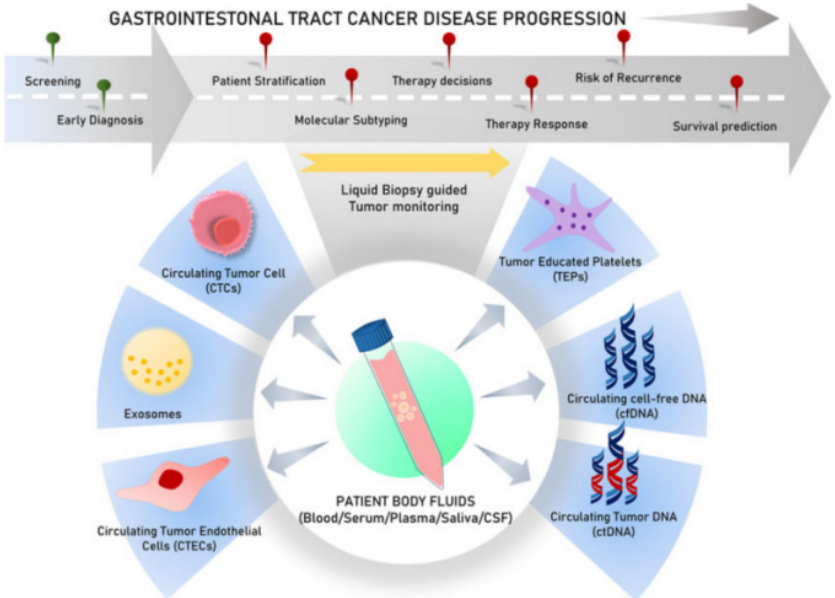

Figure 1. Schematic of various analytes used in liquid biopsy for diverse clinical applications [14]

Driven by rapid advancements in cell separation and genetic testing technologies, liquid biopsy has gained attention in oncology as a non-invasive diagnostic tool. This technology non-invasively collects bodily fluid samples such as blood, urine, and saliva to analyze tumor-derived biomarkers including circulating nucleic acids (cfDNA and cfRNA), circulating tumor cells (CTC), extracellular vesicles (EV), and tumor-induced platelets (TEP)[10]. It offers distinct advantages such as minimal invasiveness, convenient sampling, dynamic monitoring capability, and the ability to overcome tumor heterogeneity. The U.S. Food and Drug Administration (FDA) has approved multiple liquid biopsy tests, including: - Multi-marker fecal tests for colorectal cancer screening (marketed as Cologuard) and methylation-based septin 9 blood tests (marketed as Epi proColon 2.0 CE)[11]; and Guardant360® CDx and FoundationOne® Liquid CDx for non-small cell lung cancer mutation profiling[12, 13] (details available on the FDA website: https://www.fda.gov/).Notably, no FDA-approved liquid biopsy products currently exist for the specific diagnosis of HCC. Against this backdrop, platelet research—an emerging branch within liquid biopsy—is demonstrating potential to replace traditional invasive tissue biopsies and non-specific diagnostic tools, offering a new pathway for non-invasive, precise HCC diagnosis.

2. Tumor-Educated Platelets (TEPs)

Platelets, as nucleated cell fragments derived from bone marrow megakaryocytes, are not only abundant but also involved in a complex bidirectional regulatory network with the initiation and progression of hepatocellular carcinoma (HCC) (Figure 2) [27].

On one hand, platelet transport, activation, and adhesion processes are deeply involved in HCC pathological progression[15] . Platelets can indirectly drive tumor proliferation and angiogenesis by recruiting leukocytes and interacting with hepatic sinusoidal endothelial cells and hepatic stellate cells to remodel the tumor immune microenvironment [16, 17] . Additionally, tumor-associated platelets can act as physical barriers, shielding tumor cells to evade immune recognition[16]. Specifically, platelets enhance HCC cell proliferation, transendothelial migration, and epithelial-mesenchymal transition through degranulation-mediated release of bioactive substances or direct cell-to-cell contact[15, 16, 18-22] . A retrospective study involving over a thousand patients with unresectable HCC further demonstrated that thrombocytopenia significantly correlates with prolonged overall survival and reduced metastasis risk[23] .

Conversely, tumor cells can also act upon platelets, inducing their activation and aggregation while promoting the release of platelet-derived substances into the circulation[24] . For instance, HCC-derived IgG antibodies activate platelets via the FcγRIIa receptor[16]. In this bidirectional interaction, platelets continuously respond to tumor signals by actively engulfing and enriching circulating free proteins, nucleic acids, extracellular vesicles, and other biological particles. This leads to the remodeling of their RNA expression profiles and proteomic characteristics, forming TEPs ([10, 24]). Notably, the RNA composition in cancer patient platelets depends not only on the transcriptional state of megakaryocytes but is also regulated by RNA splicing events, active RNA release, and potential dynamic splicing of pre-mRNA during platelet circulation[25] .

The core value of TEPs lies in their unique capacity as biological information carriers. Benefiting from platelets' natural lifespan of 7–10 days, tumor-derived transcripts can accumulate stably within them while being effectively protected from circulating plasma RNases. This enables TEPs to comprehensively and dynamically reflect the current biological state of tumors. Combined with advantages such as high abundance, ease of acquisition, simple processing, and high standardization in detection[24 . TEP RNA demonstrates highly transformative potential in HCC liquid biopsy, particularly for early screening, precision diagnosis, and dynamic monitoring, offering a crucial direction for establishing novel non-invasive diagnostic and therapeutic systems.

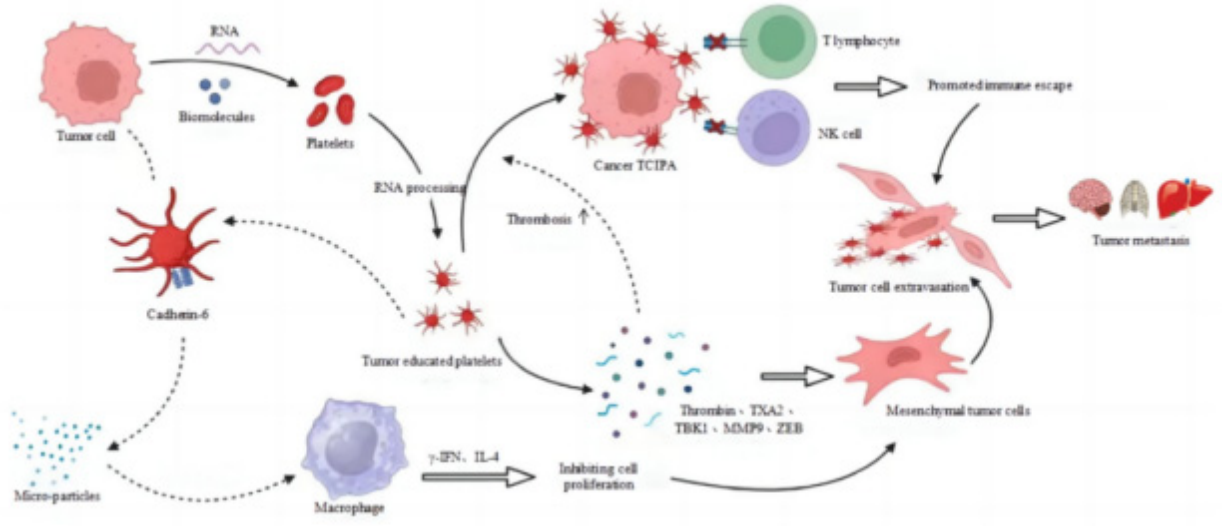

Figure 2. umor-induced platelet formation and mechanisms

3. TEP RNA

Platelets, as anucleate cells, lack DNA but are rich in diverse functional RNA molecules, including messenger RNA (mRNA), microRNA (miRNA), circular RNA (circRNA), and long non-coding RNA (lncRNA)[19] . A TEP RNA-based liquid biopsy technique reported in 2022 demonstrated up to 99% specificity in identifying 18 distinct

cancer types [27]. Furthermore, platelet RNA not only enables blood-based cancer detection but also facilitates the tracing and localization of primary tumor sites. The precise molecular pathways driving TEP RNA expression profiling remodeling remain to be elucidated; Figure 3 outlines potential regulatory mechanisms[28].

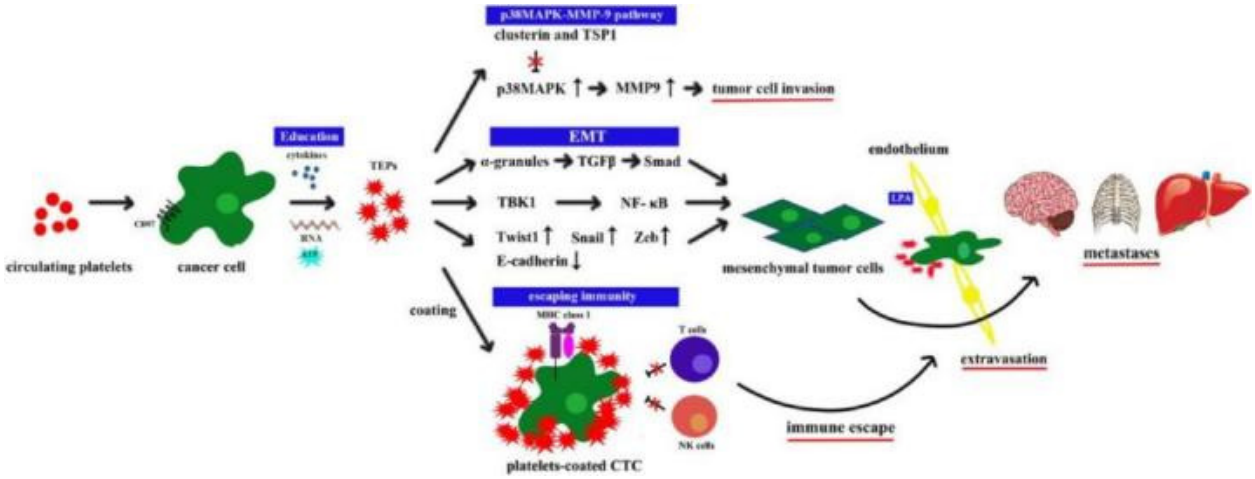

Figure 3. The potential mechanism of platelet formation in tumor education

3.1. MicroRNAs (miRNAs)

As the most abundant RNA in TEPs, miRNAs function as post-transcriptional negative regulators of gene expression by binding to the 3'-untranslated region (3'-UTR) of target mRNAs, leading to mRNA degradation or translation inhibition [29]. During tumorigenesis and progression, miRNAs promote or inhibit malignant transformation, epithelial-mesenchymal transition (EMT), and angiogenesis in tumor cells [26].

TEP miRNAs are smaller in size and inherently more stable, offering higher accuracy and specificity in tumor diagnosis. Consequently, TEP miRNAs have emerged as strong candidates for developing novel diagnostic and therapeutic approaches in oncology[26] .A bioinformatics analysis of platelet-derived miRNAs revealed significantly downregulated miR-495-3p and significantly upregulated miR-1293 in HCC patients, with receiver operating characteristic (ROC) curve areas under the curve (AUC) of 0.76 and 0.78, respectively [30].Functional studies indicate that miR-495-3p deficiency or inhibition promotes malignant transformation and proliferation in gastric cancer cells. However, the clinical-pathological significance of miR-1293 in HCC and its impact on prognosis remain to be elucidated[29] .

miRNAs serve as critical diagnostic and prognostic tools for HCC risk stratification, early detection, and treatment. In 2012, Academician Fan Jia's team identified a diagnostic model comprising eight miRNAs (hsa-miR-122, hsa-miR-192, hsa-miR-21, hsa-miR-223,hsa-miR-26a, hsa-miR-27a, hsa-miR-801, and hsa-miR-1228) from plasma (Patent Publication No.: CN102776185A), enabling early HCC diagnosis with only 2mL of plasma. Neneng et al. emphasized that miRNA-21 and miRNA-155 may serve as potential biomarkers for HCC patients, while miRNA-29c may function as a tumor suppressor[31] .Further research revealed that a combined detection model comprising miRNA-122-5p, miRNA-486-5p, and miRNA-142-3p demonstrated exceptional efficacy in distinguishing HCC from cirrhosis, achieving an AUC of 0.94,sensitivity of 80%, and specificity of 95% ($P < 0.001$), significantly outperforming alpha-fetoprotein (AUC = 0.64, P = 0.065)[32] . Given platelets as the primary source of circulating miRNAs, TEP miRNAs are also considered potential diagnostic markers for HCC[26] .

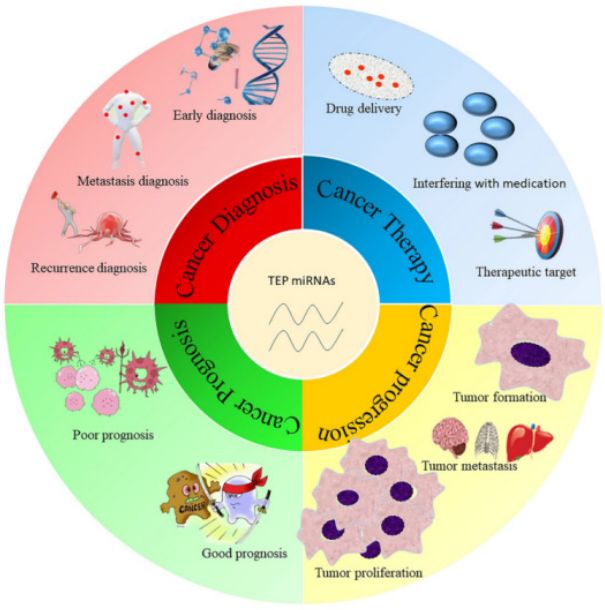

Figure 4. The role of TEP miRNA in tumor

3.2. Messenger RNA (mRNA)

mRNA is the most extensively studied RNA type in platelets, with approximately one-third of human genes (5,000–9,000 genes) identified as mRNAs in platelets[24] .In 2015, Best et al. performed mRNA sequencing analysis on 283 platelet samples, distinguishing patients with localized tumors and metastatic tumors from healthy individuals with 96% accuracy. They also differentiated six common solid tumor types (including hepatobiliary cancer, non-small cell lung cancer, colorectal cancer, glioblastoma, pancreatic cancer, and breast cancer), validating the feasibility and universality of TEP RNA for pan-cancer diagnosis [25].

Further studies indicate that specific mRNA expression patterns in TEP hold potential for early HCC detection. For instance, mRNA biomarkers such as CTNNB1, Rho A, SPINK1, and IFITM3 can aid in identifying early HCC within nodules in cirrhotic livers. This finding was validated in a cohort of 50 subjects encompassing healthy individuals, HCC patients, and cirrhotic patients[33]. Furthermore, a

preliminary exploratory study (sample size: 20 HCC patients and 10 controls) suggested that elevated mRNA expression of TGF-ß, NF-κB, and VEGF in HCC patients' TEPs may indicate disease progression to advanced stages, while decreased AKT and PIK3 levels may reflect early HCC status [22]. These findings provide preliminary evidence for HCC staging based on TEP mRNA.

3.3. Small Nucleolar RNA (SnoRNA)

SnoRNAs are a class of endogenous non-coding RNAs ranging from 60 to 200 nucleotides in length, characterized by abundant expression and high stability. These RNAs play a crucial role in ribosome biosynthesis and RNA processing by directing 2′-O-ribosyl methylation and pseudouridylation modifications of ribosomal RNA (rRNA) and small nuclear RNA (snRNA). They regulate HCC development by modulating multiple molecular signaling pathways. Therefore, snoRNAs represent potential molecular targets for HCC.

Despite being anucleate cells, platelets retain functional snoRNAs internally. These molecules participate in regulating platelet biological functions and related parameter changes by influencing the alternative splicing of precursor mRNAs [34, 35]. A study systematically analyzed the snoRNA expression profiles in the TEP of hepatitis B virus-related hepatocellular carcinoma (HBV-related HCC) using quantitative PCR technology. It revealed that SNORD12B and SNORD14E expression was significantly downregulated, while SNORA63 was markedly upregulated. These molecules demonstrated good diagnostic performance in distinguishing early-stage HBV-HCC patients from healthy individuals, with receiver operating characteristic curve areas under the curve (AUC) of 0.8386 (SNORD12B), 0.6709 (SNORA63), and 0.6826 (SNORD14E) respectively[34] .

3.4. Splicosomal Small Nucleolar RNA (SnRNA)

SnRNA is a key molecule regulating platelet RNA splicing. By governing the splicing intensity of precursor mRNA, it influences the final gene expression products. Endogenous variations in its levels can trigger widespread alternative splicing events, thereby driving the onset and progression of tumor diseases[24] .

TEPs are rich in intact, functional snRNAs whose splicing regulatory activity is preserved. Recent high-throughput sequencing studies indicate that platelets contain diverse snRNAs, including U1, U2, U6, and U12.Among these, derivative fragments of U2 snRNA (e.g., RNU2-1f) exhibit differential expression between tumor tissues (e.g., pancreatic cancer, colorectal cancer) and normal tissues. This differential expression is detectable in bodily fluids such as serum, plasma, and cerebrospinal fluid, suggesting its potential as a novel liquid biopsy biomarker. snRNA U5E-1 (RNU5E-1), a novel variant of U5 snRNA, has been shown to be downregulated in HCC, potentially serving as a diagnostic and prognostic indicator for HCC patients[36] .

Although direct research data on TEP-derived snRNA for HCC diagnosis is still accumulating, its successful exploration as a novel biomarker in other tumor types such as lung cancer[37] provides strong references and clues for snRNA-related research in HCC.

4. Conclusion

TEP RNA expression profiling offers a minimally invasive, highly sensitive new tool for early HCC diagnosis. Mechanistically, TEP formation involves multi-level biological processes including bidirectional tumor-platelet signaling, active RNA uptake and splicing regulation, and HCC microenvironment-mediated remodeling of bone marrow hematopoiesis. From a diagnostic application perspective, detection methods based on miRNA combinations and mRNA signature profiles have demonstrated superior performance to traditional biomarkers in clinical validation. From a clinical implementation standpoint, TEP detection can be integrated throughout the entire management cycle of HCC, including early screening, pathological classification, treatment efficacy assessment, and recurrence prediction.

Despite significant advances in TEP RNA research, its clinical translation faces several challenges. First, standardized workflows for TEP RNA extraction, sequencing, and analysis require harmonization. Second, existing studies predominantly rely on single-center, small-sample cohorts, necessitating validation of reproducibility through multicenter prospective trials. Additionally, the potential impact of tumor heterogeneity and coexisting liver diseases like cirrhosis on TEP RNA profiles warrants further investigation. Future research should focus on developing more stable RNA panels and integrating multidimensional liquid biopsy markers, such as circulating tumor DNA (ctDNA) and exosomal RNA, to establish more authoritative integrated diagnostic models.

References

[1] Bray, F., et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2024. 74(3): p. 229-263.

[2] Polpichai, N., et al., Current and new strategies for hepatocellular carcinoma surveillance. Gastroenterol Rep (Oxf), 2025. 13: p. goaf045.

[3] Reig, M., et al., BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. J Hepatol, 2022. 76(3): p. 681-693.

[4] Wang, Z., et al., Precision diagnosis of hepatocellular carcinoma. Chin Med J (Engl), 2023. 136(10): p. 1155-1165.

[5] Tzartzeva, K., et al., Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology, 2018. 154(6): p. 1706-1718.e1.

[6] Renzulli, M., et al., The Feasibility of Liver Biopsy for Undefined Nodules in Patients under Surveillance for Hepatocellular Carcinoma: Is Biopsy Really a Useful Tool? J Clin Med, 2022. 11(15).

[7] Yu, N.C., et al., CT and MRI improve detection of hepatocellular carcinoma, compared with ultrasound alone, in patients with cirrhosis. Clin Gastroenterol Hepatol, 2011. 9(2): p. 161-7.

[8] Teufel, A., et al., Current Trends and Advancements in the Management of Hepatocellular Carcinoma. Dig Dis, 2024. 42(4): p. 349-360.

[9] Golfieri, R., et al., Focal lesions in the cirrhotic liver: their pivotal role in gadoxetic acid-enhanced MRI and recognition by the Western guidelines. Dig Dis, 2014. 32(6): p. 696-704.

[10] Coppola, C.A., et al., Liquid Biopsy: The Challenges of a Revolutionary Approach in Oncology. Int J Mol Sci, 2025. 26(11).

[11] Loktionov, A., Biomarkers for detecting colorectal cancer non-invasively: DNA, RNA or proteins? World J Gastrointest Oncol, 2020. 12(2): p. 124-148.

[12] Kitagawa, S. and M. Seike, Liquid biopsy in lung cancer. Jpn J Clin Oncol, 2025. 55(5): p. 453-458.

[13] Kitagawa, S. and M. Seike, Liquid biopsy in lung cancer. Jpn J Clin Oncol, 2025. 55(5): p. 453-458.

[14] Mondal, D., et al., Prospects of liquid biopsy in the prognosis and clinical management of gastrointestinal cancers. Front Mol Biosci, 2024. 11: p. 1385238.

[15] Malehmir, M., et al., Platelet GPIbalpha is a mediator and potential interventional target for NASH and subsequent liver cancer. Nat Med, 2019. 25(4): p. 641-655.

[16] Mussbacher, M., et al., Till Death Do Us Part-The Multifaceted Role of Platelets in Liver Diseases. Int J Mol Sci, 2021. 22(6).

[17] Palacios-Acedo, A.L., et al., Platelets, Thrombo-Inflammation, and Cancer: Collaborating With the Enemy. Front Immunol, 2019. 10: p. 1805.

[18] Zhang, Q., X. Song and X. Song, Contents in tumor-educated platelets as the novel biosource for cancer diagnostics. Front Oncol, 2023. 13: p. 1165600.

[19] Wang, Y., et al., TEP RNA: a new frontier for early diagnosis of NSCLC. J Cancer Res Clin Oncol, 2024. 150(2): p. 97.

[20] Wang, Y., et al., Application of tumor-educated platelets as new fluid biopsy markers in various tumors. Clin Transl Oncol, 2023. 25(1): p. 114-125.

[21] Lu, M., et al., Platelets promote primary hepatocellular carcinoma metastasis through TGF-beta1-mediated cancer cell autophagy. Cancer Lett, 2024. 600: p. 217161.

[22] Asghar, S., et al., Tumor educated platelets, a promising source for early detection of hepatocellular carcinoma: Liquid biopsy an alternative approach to tissue biopsy. Clin Res Hepatol Gastroenterol, 2020. 44(6): p. 836-844.

[23] Scheiner, B., et al., Association of Platelet Count and Mean Platelet Volume with Overall Survival in Patients with Cirrhosis and Unresectable Hepatocellular Carcinoma. Liver Cancer, 2019. 8(3): p. 203-217.

[24] Ding, S., X. Dong and X. Song, Tumor educated platelet: the novel BioSource for cancer detection. Cancer Cell Int, 2023. 23(1): p. 91.

[25] Best, M.G., et al., RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics. Cancer Cell, 2015. 28(5): p. 666-676.

[26] Wang, Y., et al., Utilization of TEP miRNAs in tumor proliferation, diagnostic evaluation, therapeutic intervention, and prognostic assessment. Mol Biol Rep, 2025. 52(1): p. 343.

[27] In T Veld, S.G.J.G., et al., Detection and localization of early- and late-stage cancers using platelet RNA. Cancer Cell, 2022. 40(9): p. 999-1009.e6.

[28] Liu, L., et al., Tumor-educated platelet as liquid biopsy in lung cancer patients. Crit Rev Oncol Hematol, 2020. 146: p. 102863.

[29] Eun, J.W., et al., MicroRNA-495-3p functions as a tumor suppressor by regulating multiple epigenetic modifiers in gastric carcinogenesis. J Pathol, 2018. 244(1): p. 107-119.

[30] Zhu, B., et al., Bioinformatics analysis of tumor-educated platelet microRNAs in patients with hepatocellular carcinoma. Biosci Rep, 2021. 41(12).

[31] Ratnasari, N., et al., Potential plasma biomarkers: miRNA-29c, miRNA-21, and miRNA-155 in clinical progression of Hepatocellular Carcinoma patients. PLoS One, 2022. 17(2): p. e0263298.

[32] Weis, A., et al., Serum MicroRNAs as Biomarkers in Hepatitis C: Preliminary Evidence of a MicroRNA Panel for the Diagnosis of Hepatocellular Carcinoma. Int J Mol Sci, 2019. 20(4).

[33] Waqar, W., S. Asghar and S. Manzoor, Platelets' RNA as biomarker trove for differentiation of early-stage hepatocellular carcinoma from underlying cirrhotic nodules. PLoS One, 2021. 16(9): p. e0256739.

[34] Zhao, X., et al., TEP SNORD12B, SNORA63, and SNORD14E as novel biomarkers for hepatitis B virus-related hepatocellular carcinoma (HBV-related HCC). Cancer Cell Int, 2024. 24(1): p. 3.

[35] Wang, H., et al., Small nucleolar RNA U2_19 promotes hepatocellular carcinoma progression by regulating Wnt/beta-catenin signaling. Biochem Biophys Res Commun, 2018. 500(2): p. 351-356.

[36] Ding, Y., et al., Down-regulation of small nuclear RNA (snRNA) RNU5E-1 in hepatocellular carcinoma presents with vital clinical significance. J Gastrointest Oncol, 2020. 11(4): p. 738-746.

[37] Dong, X., et al., Small Nuclear RNAs (U1, U2, U5) in Tumor-Educated Platelets Are Downregulated and Act as Promising Biomarkers in Lung Cancer. Front Oncol, 2020. 10: p. 1627.