Current Pharmaceutical Letters and Reviews (CPLR)
Website: https://cplr.in/
ISSN: 3049-222X
Vol. 2, Issue 3, July-Sept, 2025
Page No.: 30-36

Review

Exosome-Derived Biomarkers in Carcinogenesis and Tumor Microenvironment Communication

Prafulkumar Rajendrabhai Suryavanshi
Post Graduate Scholar, Parul Institute of Ayurved and Research, Parul University, PO: Ishwarpura, Ta:Waghodia, Dist: Vadodara, Pin: 391760

Corresponding Author:
Dr Prafulkumar Rajendrabhai Suryavanshi

Email:
drprafulsuryavanshi@gmail.com

DOI: *10.62896/cplr.2.3.05*

Conflict of interest: *NIL*

Article History
Received: 12/07/2025
Accepted: 28/08/2025
Published: 10/09/2025

Abstract:
Exosomes, small vesicles secreted by various cell types, have garnered significant attention in recent years for their role in intercellular communication. As key mediators of the tumor microenvironment (TME), exosomes are involved in multiple aspects of carcinogenesis, including immune evasion, metastasis, and tumor progression. They are enriched with biomolecules, including proteins, lipids, RNA, and DNA, that reflect the pathological state of their cell of origin, making them promising candidates for non-invasive biomarkers in cancer diagnosis, prognosis, and therapeutic monitoring. This paper explores the emerging roles of exosome-derived biomarkers in carcinogenesis and their impact on the TME, highlighting their potential utility in clinical applications. We provide an in-depth analysis of the molecular signatures carried by exosomes from tumor cells and their influence on surrounding stromal cells, immune cells, and the extracellular matrix. Additionally, we discuss the current challenges in utilizing exosome-based biomarkers and outline future directions for enhancing their diagnostic and therapeutic value in oncology.

Keywords: Exosomes, Tumor Microenvironment, Carcinogenesis, Biomarkers, Tumor Progression, Immune Evasion, Metastasis, Extracellular Vesicles, Cancer Diagnosis, Therapeutic Monitoring.

1.1 Introduction:

Cancer is a complex and multifaceted disease characterized by uncontrolled cell growth, invasion, and metastasis. The tumor microenvironment (TME), which consists of a dynamic network of stromal cells, immune cells, blood vessels, and extracellular matrix components, plays a crucial role in the initiation, progression, and spread of tumors. Recent advances in cancer research have highlighted the significant contribution of exosomes—small, membrane-bound extracellular vesicles—as key mediators of intercellular communication within the TME. Exosomes are secreted by virtually all cell types, including cancer cells, and are known to carry a diverse range of bioactive molecules, such as proteins, lipids, RNAs, and DNA, which can influence the behavior of recipient cells.(1)

These exosome-derived cargo molecules reflect the molecular and genetic landscape of their origin cells, making them potential biomarkers for early cancer detection, prognosis, and monitoring therapeutic responses. Notably, exosomes can facilitate tumorigenesis by modulating immune responses, promoting angiogenesis, enhancing metastatic potential, and reshaping the TME to support cancer cell survival. Their ability to traverse biological barriers and carry complex molecular signatures offers a unique, non-invasive approach for understanding tumor biology and developing personalized cancer therapies.

Despite the promising potential of exosomes as biomarkers, several challenges remain in their clinical application. The heterogeneity of exosomes, variations in their cargo depending on tumor type, and difficulties in isolating and characterizing them from biological fluids complicate their use in routine clinical settings. Nevertheless, ongoing research into the molecular mechanisms underlying exosome

biogenesis and their interactions with the TME is paving the way for the development of novel diagnostic tools and therapeutic strategies.(2)

This paper aims to provide a comprehensive overview of exosome-derived biomarkers in carcinogenesis, their role in shaping the tumor microenvironment, and their emerging applications in cancer diagnosis and treatment. By examining the current landscape and highlighting key challenges, we aim to underscore the potential of exosomes as a transformative tool in oncology.

1.2 Introduction to Cancer and Tumor Microenvironment:

Cancer is a collection of diseases characterized by uncontrolled cell growth, evasion of normal regulatory processes, and the ability to invade surrounding tissues or metastasize to distant organs. Unlike normal cells, cancer cells bypass growth controls, undergo genetic mutations, and exhibit altered cellular behaviors that allow them to thrive in abnormal environments.(3) A central feature of cancer is the tumor microenvironment (TME), which is a complex and dynamic ecosystem surrounding the tumor. The TME includes not only the cancer cells themselves but also a variety of stromal cells (fibroblasts, endothelial cells, and immune cells), blood vessels, extracellular matrix components, and signaling molecules. These components interact in a bidirectional manner, influencing tumor development and progression. The TME not only provides a supportive niche for tumor cells to grow but also plays a significant role in promoting the hallmarks of cancer, including immune evasion, angiogenesis, and metastasis. Therefore, understanding the interactions within the TME is critical to unraveling the mechanisms of carcinogenesis and developing more effective cancer therapies.(4)

1.3 The Complexity of Cancer Development and Progression:

Cancer development, also known as carcinogenesis, is a multi-step process involving genetic mutations, epigenetic alterations, and dysregulated signaling pathways that collectively lead to malignant transformation. Carcinogenesis typically begins with a genetic mutation in a single cell, which causes uncontrolled cell division.(5) Over time, additional mutations accumulate, enabling cancer cells to acquire characteristics such as sustained proliferative signaling, resistance to cell death, and the ability to invade surrounding tissues. As cancer progresses, the tumor evolves through continuous genetic and phenotypic changes, adapting to environmental pressures, such as hypoxia, nutrient deprivation, and immune surveillance. This evolutionary process also leads to the selection of more aggressive cancer cells with increased metastatic potential. Tumor progression is not only driven by the inherent properties of the cancer cells but also heavily influenced by the TME, which can alter cellular behavior through complex signaling interactions. The ability of tumors to manipulate their environment to their advantage is one of the key factors that make cancer development so complex and difficult to treat.(6)

1.4 The Role of Tumor Microenvironment in Carcinogenesis:

The tumor microenvironment plays a pivotal role in carcinogenesis by influencing both the initiation and progression of cancer. Far from being a passive backdrop, the TME actively interacts with cancer cells and shapes their behavior through numerous mechanisms. One of the primary functions of the TME is to provide a favorable environment for tumor cell survival and growth. (7)Tumor-associated fibroblasts, immune cells, and endothelial cells release growth factors, cytokines, and extracellular matrix components that support tumor cell proliferation and tissue invasion. Additionally, the TME can promote immune evasion, allowing tumor cells to escape detection and destruction by the body's immune system. Tumor cells can also reprogram normal stromal cells, creating a supportive niche that aids in tumor growth and metastasis. Moreover, the TME is involved in processes such as angiogenesis, the formation of new blood vessels, which ensures an adequate supply of oxygen and nutrients to support rapid tumor growth. Overall, the TME is not just a passive bystander but an active participant in the development of cancer, influencing the tumor's ability to progress, metastasize, and resist treatment.(8)

1.5 Exosomes: Key Players in Intercellular Communication

Exosomes are small, membrane-bound vesicles that are secreted by a wide variety of cell types, including cancer cells, and play a critical role in intercellular communication. They serve as carriers of information between cells, mediating the transfer of bioactive molecules such as proteins, lipids, RNA, and DNA.(9) This intercellular communication

allows exosomes to influence the behavior of recipient cells, thereby impacting various physiological processes, including immune responses, tissue repair, and even cancer progression. In the context of cancer, exosomes contribute to the complex interactions within the tumor microenvironment (TME), facilitating tumor growth, metastasis, and immune evasion. They enable tumor cells to modulate the surrounding stroma, recruit immune cells, and reprogram endothelial cells and fibroblasts, thereby shaping the TME in a way that favors tumor progression. Exosomes also help cancer cells to communicate with distant sites, promoting metastasis by carrying signals that enable colonization of new tissues. Thus, exosomes are emerging as crucial mediators of tumor biology and as potential biomarkers for cancer diagnosis and therapeutic monitoring.(10)

1.6 Exosome Biogenesis and Composition

Exosome biogenesis begins with the inward budding of the plasma membrane, forming early endosomes. These endosomes undergo maturation to late endosomes, which eventually form multivesicular bodies (MVBs). Within MVBs, smaller vesicles are encapsulated, which are then released into the extracellular space upon fusion of the MVBs with the plasma membrane. (11)The process of exosome release is tightly regulated and involves the action of various proteins, including tetraspanins (such as CD63, CD9, and CD81), the ESCRT (Endosomal Sorting Complex Required for Transport) machinery, and Rab GTPases, which control the trafficking of vesicles. The composition of exosomes is highly heterogeneous and depends on the cell type and the specific physiological or pathological context. They contain a wide range of bioactive molecules, including lipids, proteins, mRNA, and non-coding RNAs, which are selectively packaged into exosomes during their biogenesis. This selective packaging is mediated by specific sorting mechanisms that ensure the incorporation of cargo relevant to the functional role of the exosome. The exosome membrane itself is composed primarily of phospholipids, and their surface proteins are involved in interactions with recipient cells, allowing for the delivery of their cargo to target cells. Exosome biogenesis is a critical process that not only dictates the composition of the vesicles but also regulates their biological function in health and disease.(12)

Exosomes carry a diverse and complex cargo that reflects the functional state and molecular characteristics of their cell of origin. The cargo includes proteins, lipids, RNA (including mRNA, microRNA, and long non-coding RNA), and DNA, each playing a specific role in the intercellular communication mediated by exosomes.

Proteins: Exosomal proteins are involved in various functions, such as membrane trafficking, cell signaling, and immune modulation. Tetraspanins, integrins, and other membrane-associated proteins play a crucial role in the uptake of exosomes by recipient cells, while signaling molecules like growth factors and cytokines can alter the behavior of recipient cells. Proteins involved in the ESCRT machinery and molecular chaperones are also present, ensuring proper exosome formation and cargo selection.

Lipids: The lipid composition of exosomes is similar to that of the parent cell membrane but also contains distinct lipid species that facilitate exosome stability, fusion with recipient cells, and modulation of cellular processes. Specific lipids, such as ceramide and sphingomyelin, play an essential role in the biogenesis of exosomes and in mediating interactions with recipient cells.

RNA: Exosomes carry a variety of RNA species, including messenger RNAs (mRNA), microRNAs (miRNAs), and long non-coding RNAs (lncRNAs). These RNA molecules can be delivered to recipient cells, where they may influence gene expression, protein synthesis, and cellular functions. miRNAs, in particular, are known to regulate gene expression by binding to complementary mRNA targets and are often involved in modulating processes like cell proliferation, apoptosis, and metastasis.(13)

DNA: Although less studied than RNA, exosomes can also carry DNA fragments, including both nuclear and mitochondrial DNA. These DNA fragments may have functional implications in disease processes, such as in tumorigenesis, where tumor-derived exosomal DNA can potentially contribute to genetic mutations in recipient cells, influencing their behavior and contributing to tumor progression.

1.7 Exosomes and Immune Evasion in Cancer

One of the key strategies by which tumors evade immune surveillance is through the modulation of the immune system, and exosomes play a crucial role in this process. Cancer cells secrete exosomes that carry molecules capable of suppressing immune

responses, thereby enabling the tumor to escape detection and destruction by the host's immune system. These exosomes can carry immunosuppressive proteins, cytokines, and microRNAs that directly inhibit immune cell activity. For example, tumor-derived exosomes often contain proteins like programmed death-ligand 1 (PD-L1), which interacts with immune checkpoint receptors on T cells, leading to T cell exhaustion and immune evasion. Exosomes can also carry microRNAs that suppress the function of immune cells such as dendritic cells, macrophages, and natural killer (NK) cells, further impairing the immune response. Additionally, tumor exosomes can promote the recruitment of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which further dampen anti-tumor immunity. Through these mechanisms, exosomes help tumors create an immune-suppressive environment that enables continued growth, metastasis, and resistance to treatment.(14)

1.8 Exosomes in Tumor Metastasis and Angiogenesis

Exosomes also play a critical role in tumor metastasis and angiogenesis, two processes that are vital for cancer progression. Metastasis involves the spread of cancer cells from the primary tumor to distant organs, a process facilitated by the TME. Exosomes contribute to metastasis by promoting the epithelial-to-mesenchymal transition (EMT), a cellular process that enables cancer cells to acquire migratory and invasive properties.(15) Exosome cargo, such as specific proteins and microRNAs, can activate signaling pathways that promote EMT in recipient tumor cells, enhancing their ability to invade surrounding tissues and travel through the bloodstream to distant sites. Exosomes can also influence the behavior of endothelial cells, facilitating the formation of new blood vessels in the process known as angiogenesis. Tumor-derived exosomes contain pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), that stimulate endothelial cells to form new blood vessels, thereby improving the tumor’s ability to obtain nutrients and oxygen. Furthermore, exosomes can modulate the extracellular matrix (ECM), creating a more permissive environment for tumor cell migration and metastasis. By promoting both metastasis and angiogenesis, exosomes help tumors grow, spread, and adapt to the challenges of distant tissue environments.(16)

1.9 Exosome-Mediated Modulation of the Tumor Microenvironment

Exosomes are central to the dynamic and reciprocal interactions between tumor cells and their surrounding tumor microenvironment (TME). The TME consists of cancer cells, stromal cells (e.g., fibroblasts, endothelial cells), immune cells, and extracellular matrix components, all of which influence tumor growth and progression. Exosomes secreted by cancer cells communicate with these various TME components, effectively modulating their functions to create a supportive niche for tumor growth. For example, exosomes from tumor cells can activate fibroblasts to secrete pro-tumorigenic cytokines and extracellular matrix proteins that promote tumor cell survival and invasion.(17) Tumor exosomes can also interact with endothelial cells to enhance angiogenesis and increase vascular permeability, providing more oxygen and nutrients to support tumor expansion. Furthermore, exosomes influence the immune cells within the TME by promoting immune suppression or reprogramming immune cells to adopt pro-tumor functions. By transferring molecules that modify the activity of stromal and immune cells, exosomes help create a TME that is more conducive to tumor progression. Exosome-mediated modulation of the TME is a critical aspect of cancer biology, as it not only enhances tumor growth and metastasis but also contributes to therapeutic resistance, making exosomes important targets for novel cancer therapies.(18)

1.10 Potential of Exosome-Derived Biomarkers in Cancer Diagnosis

Exosome-derived biomarkers have shown significant promise in the early detection and diagnosis of cancer. Due to their ability to encapsulate and protect various biomolecules, such as proteins, lipids, RNAs, and DNA, exosomes reflect the molecular characteristics of their originating cells.(19) Tumor-derived exosomes, in particular, carry specific biomarkers that can indicate the presence of cancer, making them valuable for non-invasive diagnostic testing. For instance, exosomes can be isolated from body fluids such as blood, urine, and saliva, offering a minimally invasive method for monitoring cancer biomarkers. The molecular cargo within these exosomes can provide a comprehensive snapshot of the tumor’s

Current Pharmaceutical Letters and Reviews (CPLR)
Website: https://cplr.in/
ISSN: 3049-222X
Vol. 2, Issue 3, July-Sept, 2025
Page No.: 30-36

genetic and proteomic profile, including mutations, gene expression patterns, and protein signatures, which can aid in identifying cancer types, stages, and even predicting the likelihood of metastasis. The potential of exosomes as biomarkers lies in their ability to detect tumors at early stages, often before clinical symptoms arise, providing an opportunity for early intervention and improved patient outcomes. However, further research is needed to standardize exosome-based diagnostic methods and to refine their sensitivity and specificity for different cancer types.(20)

1.11 Exosomes as Prognostic Biomarkers in Oncology

Exosomes are not only useful for cancer diagnosis but also hold significant potential as prognostic biomarkers. By analyzing the molecular contents of exosomes, clinicians can gain valuable insights into the progression and behavior of a tumor. The composition of exosomes can reflect key aspects of tumor biology, such as aggressive behavior, metastasis, and response to treatment.(21) For example, the presence of certain proteins, such as PD-L1 or various oncogenes, within exosomes can provide information on the tumor's ability to evade immune detection or its potential for growth and spread. Additionally, exosomal microRNAs have been identified as important regulators of cancer progression, with specific miRNAs linked to poor prognosis or resistance to therapies. By monitoring changes in exosome profiles over time, clinicians may be able to track disease progression, assess the effectiveness of treatment, and predict patient outcomes. Exosome-based prognostic biomarkers could thus be used to tailor individualized treatment plans, allowing for more personalized and effective care. However, clinical validation of these biomarkers is still necessary to establish their reliability and predictive value across different patient populations and cancer types.(22)

1.12 Challenges in Isolating and Characterizing Exosomes

While exosomes hold great promise as biomarkers for cancer diagnosis and prognosis, several challenges remain in isolating and characterizing them. Exosomes are small (30–150 nm in diameter), and their isolation requires highly sensitive techniques that can separate them from other extracellular vesicles or cellular debris present in biological samples. Current methods for exosome isolation, such as ultracentrifugation, size exclusion chromatography, and immunoaffinity capture, can be time-consuming, complex, and prone to contamination, leading to variability in results.(23) Furthermore, the heterogeneity of exosomes poses another challenge. Exosomes vary in size, content, and surface markers depending on the cell type and physiological or pathological conditions from which they originate. This diversity makes it difficult to standardize isolation and characterization protocols. Additionally, the molecular cargo within exosomes—proteins, RNA, and DNA—can be present in varying concentrations, and identifying specific markers linked to particular cancers remains a challenge. Advanced analytical techniques, such as high-throughput sequencing, mass spectrometry, and single-particle tracking, are required to accurately profile exosome contents, but these methods often require specialized equipment and expertise. Overcoming these technical challenges is essential to advancing the clinical utility of exosomes as reliable biomarkers in cancer.(24)

1.13 Current Strategies for Clinical Application of Exosome-Based Biomarkers

Despite the challenges, there are ongoing efforts to translate exosome-based biomarkers into clinical practice. Several strategies are being developed to enable the use of exosomes in cancer diagnostics and treatment monitoring. One promising approach involves developing more efficient, cost-effective, and standardized techniques for exosome isolation and characterization, making their use in routine clinical settings more feasible. Liquid biopsy technologies, which involve the detection of exosomes in bodily fluids like blood, urine, or saliva, are at the forefront of these efforts.(25) Liquid biopsies provide a non-invasive alternative to tissue biopsies, allowing for real-time monitoring of tumor dynamics and molecular alterations. In clinical trials, exosome-based liquid biopsies have been shown to detect mutations, gene expression changes, and protein markers that are indicative of specific cancer types, stages, and therapeutic responses. Furthermore, advancements in exosome engineering—such as the modification of exosomes to carry therapeutic agents or the development of exosome-based vaccines—are opening up new possibilities for targeted cancer therapies. Clinical studies are also exploring the potential of exosome-based biomarkers for predicting therapeutic outcomes, assessing treatment resistance, and monitoring disease relapse. As research progresses,

Current Pharmaceutical Letters and Reviews (CPLR)
Website: https://cplr.in/
ISSN: 3049-222X
Vol. 2, Issue 3, July-Sept, 2025
Page No.: 30-36

the integration of exosome-based technologies into clinical practice holds the potential to revolutionize cancer diagnosis, prognosis, and treatment strategies, offering a more personalized and less invasive approach to cancer care.(26)

Exosome-Derived Biomarker	Role in Carcinogenesis	Tumor Microenvironment Impact
PD-L1 (Programmed Death-Ligand 1)	Promotes immune evasion by inhibiting T-cell activation	Reprograms immune cells, induces immune suppression
miR-21	Regulates cell proliferation, migration, and survival, contributing to tumorigenesis	Promotes angiogenesis and immune evasion
miR-10b	Facilitates metastasis and invasion, promoting tumor progression	Enhances cell migration and invasion in the TME
EGFRvIII	Mutant form is involved in the aggressive growth of glioblastoma	Modifies the TME to support tumor cell survival
VEGF (Vascular Endothelial Growth Factor)	Stimulates angiogenesis, promoting tumor vascularization	Facilitates endothelial cell function in angiogenesis
TGF-Î² (Transforming Growth Factor Beta)	Induces immunosuppressive environment, promotes tumor progression	Regulates immune cell trafficking and extracellular matrix remodeling
MMP-9 (Matrix Metalloproteinase-9)	Facilitates ECM degradation and promotes invasion	Modulates the extracellular matrix and stromal cells
CD63 (Tetraspanin Protein)	Facilitates exosome uptake and contributes to cell-cell communication in cancer	Interacts with immune cells and endothelial cells, promoting tumor progression
HER2 (Human Epidermal Growth Factor Receptor 2)	Overexpression promotes tumor growth and metastasis	Promotes immune evasion and tumor cell survival in the TME
miR-155	Regulates immune response and tumor progression	Affects inflammatory responses, contributing to tumor growth

CONCLUSION:

The advent of liquid biopsy, particularly through the analysis of circulating tumor DNA (ctDNA), represents a paradigm shift in cancer diagnostics and treatment. Unlike traditional biopsy methods, liquid biopsy offers a non-invasive, accessible, and real-time approach to detecting and monitoring cancer, allowing for earlier detection, continuous assessment, and personalized treatment strategies. ctDNA, as a molecular marker, provides valuable insights into tumor mutations, genetic alterations, and tumor evolution, enhancing our understanding of cancer biology and its heterogeneity. It plays a crucial role not only in early cancer detection but also in monitoring minimal residual disease and evaluating treatment responses, which are essential for preventing relapse and improving long-term patient outcomes.

While challenges remain, including issues related to sensitivity, specificity, and standardization of methods, the clinical application of ctDNA is rapidly advancing. The ability to monitor cancer progression without the need for invasive procedures presents a significant advantage, making it a powerful tool in precision oncology. As research continues to unfold and technologies improve, ctDNA-based liquid biopsy is poised to become a cornerstone in the future of cancer care, offering more effective, less invasive, and more personalized approaches to treatment. Ultimately, the integration of ctDNA analysis into routine clinical practice has the potential to revolutionize cancer diagnosis, treatment, and monitoring, ultimately leading to better patient outcomes and a more dynamic approach to cancer management.

REFERENCES:

1. Alix-Panabières C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. *Clin Chem*. 2013;59(1):110-8.
2. Alix-Panabières C, Arnold N, Schneider R, et al. Circulating tumor cells as an

emerging diagnostic tool. *Clin Chem Lab Med*. 2017;55(2):243-53.
3. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6(224):224ra24.
4. Bruix J, Gores GJ, Llovet JM. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gastroenterology*. 2014;146(4):1087-103.
5. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science*. 2011;331(6024):1559-64.
6. Chaudhuri AA, Chabon JJ, Lovejoy AF, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. *Cancer Discov*. 2017;7(12):1394-403.
7. Cohen JD, Li L, Wang Y, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science*. 2018;359(6378):926-30.
8. Diaz LA, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*. 2012;486(7404):537-40.
9. Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. *Nat Med*. 2008;14(9):985-90.
10. Ding L, Wendl MC, Koboldt DC, et al. Expansion of cancer genome sequencing. *Cancer Cell*. 2012;22(6):601-12.
11. Dolan A, Weitzman S. Challenges in cancer early detection. *J Cancer Detect*. 2015;19(2):101-5.
12. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023-31.
13. Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med*. 2015;7(302):302ra133.
14. Garcia-Murillas I, Turner NC. Liquid biopsy for early-stage breast cancer: current evidence and future directions. *J Clin Oncol*. 2020;38(13):1415-24.
15. Garlan F, Laurent-Puig P, Sefrioui D, et al. Circulating tumor DNA as a predictive biomarker in metastatic colorectal cancer. *Ann Oncol*. 2017;28(4):601-6.
16. García-Olmo D, García-Olmo M, Sanz-Aparicio M, et al. Circulating tumor DNA as a marker of minimal residual disease in gastrointestinal cancer. *Cancer Treat Rev*. 2022;98:102235.
17. Heitzer E, Auer M, Roberts C, et al. Establishment of genomic alterations in ctDNA for precision oncology. *Mol Oncol*. 2019;13(1):10-22.
18. Hyman DM, Taylor BS, Baselga J. Implementing genome-driven oncology. *Cell*. 2017;168(4):584-99.
19. Kaps L, Hafner C, Kittel C, et al. Liquid biopsy in melanoma: state-of-the-art and future perspectives. *Eur J Cancer*. 2019;118:78-88.
20. Khakoo S, Richards R, Parker R, et al. ctDNA as a prognostic marker for early-stage cancer detection: A comprehensive review. *J Clin Oncol*. 2021;39(12):1578-86.
21. Kinde I, Wu J, Papadopoulos N, et al. Detection and quantification of rare mutations with massively parallel sequencing. *Proc Natl Acad Sci USA*. 2012;109(36):14508-13.
22. Leal A, Perez-Ruiz E, Ribelles N, et al. Liquid biopsy as a tool to assess the molecular landscape in early-stage breast cancer. *Oncologist*. 2021;26(7):558-65.
23. Luthra R, Taly V, Lee J, et al. Circulating tumor DNA as a diagnostic tool in liquid biopsy. *J Mol Diagn*. 2019;21(1):52-62.
24. Murtaza M, Dawson SJ, Tsui DW, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature*. 2013;497(7447):108-12.
25. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantifying circulating tumor DNA with broad patient coverage. *Nat Med*. 2016;22(5):548-53.
26. Pantel K, Alix-Panabières C. Liquid biopsy: potential and challenges. *Mol Oncol*. 2019;13(1):37-46.

*****