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Exosome-based liquid biopsy: A new frontier in early cancer diagnosis

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SUMMARY: Early-stage diagnosis offers the greatest survival advantage in oncology, and yet conventional liquid-biopsy markers — circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) — depend on cell death or mechanical shedding and therefore appear late in disease progression. Exosomes, 40–160 nm lipid-bilayer vesicles secreted by viable cells, emerge earlier, outnumber CTCs by several orders of magnitude, and preserve multi-omic cargo that mirrors intratumor heterogeneity. Rapid advances in enabling technologies are driving continual breakthroughs in exosome-based liquid biopsy, laying a solid foundation for its accelerated translation into clinical practice. Key hurdles remain: standardizing exosome isolation, defining quantitative cut-offs that separate malignant from inflammatory EV surges, and building probabilistic multi-omic models to pinpoint tissue origin. Eliminating these obstacles could advance detection by months and shift care from late salvage to true early interception.

Keywords: exosomes, liquid biopsy, early cancer detection

Early detection remains the most powerful lever for improving cancer survival. Contemporary Surveillance, Epidemiology, and End Results statistics show that the five year relative survival for common solid tumors exceeds 80% in stage I yet falls below 20% in stage IV (*1*). Circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) have improved monitoring in advanced disease, but both markers depend on cell death or physical shedding and therefore rise late (*2*). Exosomes, which are lipid bilayer vesicles 40 to 160 nanometers in diameter and secreted by viable cells, provide an earlier signal. They outnumber CTCs by several orders of magnitude, enclose intact RNA, DNA, protein, and lipid cargo, and capture intratumor heterogeneity (*3*) (Figure 1). In recent years, with the increasing depth of exosome research, exosome-based liquid biopsy has shown great promise as a novel strategy for the early diagnosis of cancer.

Technical barriers that once limited vesicle work have narrowed. Acoustic microfluidic fractionation now isolates exosomes directly from whole blood within minutes without labels and preserves their functional integrity (*4*). Aptamer functionalized microbead sensors detect CD63- or EpCAM-positive vesicles in two microliters of plasma with a 30-minute turnaround suited to community screening (*5*). Single vesicle imaging flow cytometry and nanoplasmonic readers routinely phenotype particles below 100 nanometers, enabling multiplex counts of PD L1, EpCAM, or glypican 1 positive subpopulations in unprocessed fluids (*6*). Cargo loading into exosomes is an active, energy-dependent process, meaning that the vesicular miRNA profile reflects real-time transcriptional programs in living tumor cells. In contrast, cfDNA fragments derive mainly from apoptosis and necrosis and therefore provide a static snapshot of historical genomic alterations. Integrating both read-outs brings genotype and dynamic pathway activity together in one test (*7,8*).

Exosomal miRNAs travel inside 40–160-nm lipid-bilayer vesicles, a structure that keeps them intact for days in plasma and even allows them to cross barriers such as the blood–brain barrier, whereas protein-bound cell-free miRNAs are exposed to RNases and typically decay within hours (*9*). Exosomes still wear the "name tags" (surface proteins) of the cells that made them. Scientists can use antibodies or nano-flow cytometry to grab vesicles with a specific tag — say, EpCAM — to isolate those coming from epithelial tumors. Cell-free miRNAs and cfDNA float naked in the blood with no membrane or protein tags, so the same trick cannot reveal their tissue of origin (*10*). Clinical data illustrate this complementarity. In a multicenter study of 292 participants, a 13-marker panel containing eight exosomal and five cell-free miRNAs was created; the composite signature detected stage I–II pancreatic ductal adenocarcinoma with an area under the curve

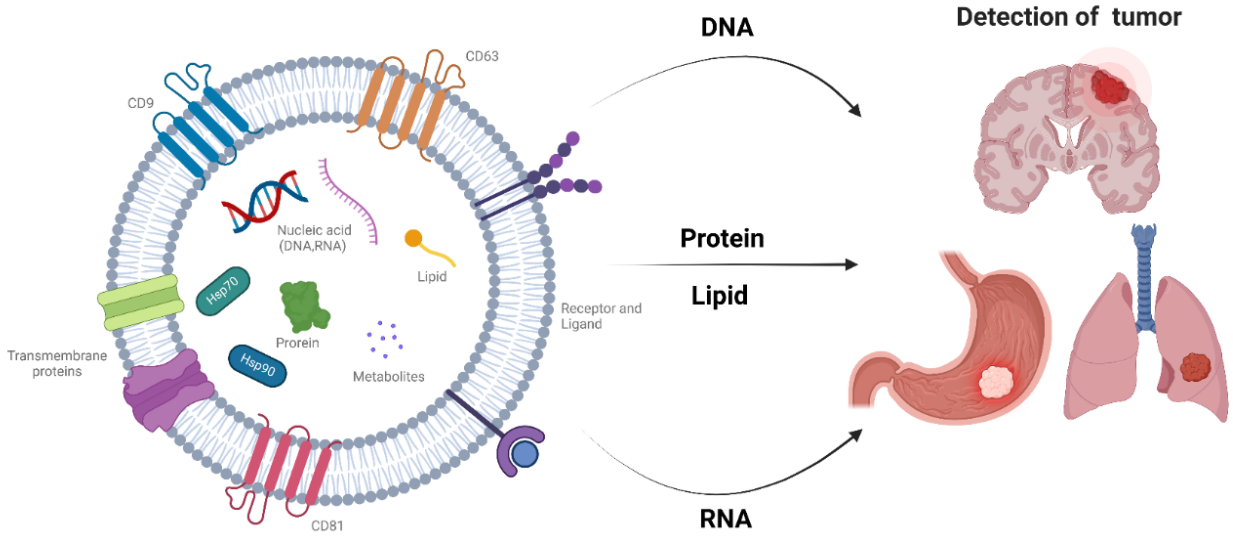

Figure 1. Schematic illustration of exosome-based tumor diagnosis. Tumor cells actively secrete large quantities of exosomes into bodily fluids such as blood, urine, and saliva. These exosomes carry tumor-specific biomarkers, including proteins, nucleic acids (DNA, mRNA, and non-coding RNAs), and lipids, which reflect the molecular and genetic characteristics of the parent tumor.

(AUC) of 0.96, outperforming either subset alone (*11*). In non-small-cell lung cancer, adding exosomal RNA to ctDNA increased the number of EGFR-activating-mutation copies almost 10-fold and raised detection sensitivity in early (M0/M1a) disease from 26% to 74%, an improvement that derives directly from combining the two analyte classes (*12*). Taken together, exosomal miRNAs offer stability, selective enrichment and live-cell transcriptomic context, while cf-miRNAs and cfDNA provide convenient access to circulating molecules and hard genomic endpoints. Capitalizing on their complementary strengths in unified, multi-analyte assays yields markedly higher sensitivity and specificity than relying on any single liquid-biopsy component.

Clinical validation is gaining pace. Glypican-1 positive exosomes distinguish pancreatic ductal adenocarcinoma from benign pancreatic disease with a sensitivity of 100% and a specificity of 100%, and their levels correlate with tumor burden and patient survival after surgical resection (*13,14*). A single-center deep-sequencing study of serum exosomes from nine stage II–IV colorectal-cancer patients and three healthy controls identified a 12-microRNA signature; six miRNAs were significantly up-regulated — four let-7 family members (let-7a-5p, let-7c-5p, let-7f-5p, and let-7d-3p) together with miR-423-5p and miR-3184-5p — and all six peaked in stage II before declining in stages III and IV, while qRT-PCR validation confirmed a > 2-fold elevation of the two representative candidates miR-423-5p and miR-3184-5p with $P < 0.05$, underscoring their promise as minimally invasive early-stage biomarkers (*15*). In a multicenter study, a fucosylated-extracellular-vesicle five-miRNA signature had a sensitivity of 90% and a specificity of 92% for hepatocellular carcinoma in a 606-subject cohort (194 with HCC, 412 with non-HCC) and significantly outperformed both α-fetoprotein and des-γ-carboxy prothrombin (*16*). The iExoDisc, developed by Zhao and colleagues, is an automated centrifugal microfluidic platform for efficiently isolating exosomes from blood samples and performing glycan analysis. The benefits of this platform include: completing exosome isolation within 45 minutes, saving significant time compared to conventional methods like ultracentrifugation; improving exosome purity by 3 to 6 times and achieving a recovery rate of 74.7%; additionally, iExoDisc can identify potential diagnostic markers, such as galactosylation and sialylation, from plasma samples of patients with triple-negative breast cancer (TNBC). This technology provides a new solution for early cancer diagnosis and liquid biopsy (*17*). Meta analyses that pool data from several tumor types assign exosomal microRNA diagnostics a mean AUC of close to 0.84 with a balanced sensitivity and specificity in early disease (*18*). Ge *et al*. identified bile-derived exosomal miR-483-5p and miR-126-3p as biomarkers for distinguishing malignant from benign biliary obstructions (*19*). RNA sequencing in 82 patients showed significant elevation of both miRNAs in malignant cases. miR-483-5p had an AUC of 0.81 (sensitivity of 81.1% and specificity of 81.1%), while miR-126-3p had an AUC of 0.74 (sensitivity of 73.0% and specificity of 86.5%), outperforming CA19-9. These findings highlight the potential of miR-483-5p and miR-126-3p as effective, non-invasive diagnostic tools for malignant biliary obstructions. Clinical translation of engineered exosome therapeutics is steadily advancing; notably, a first-in-human study (NCT03608631) of a Good-Manufacturing-Practice batch of mesenchymal-stromal-cell–derived exosomes carrying KRASG12D-targeting siRNA (iExosomes) is presently assessing safety and feasibility

in patients with pancreatic cancer (*20*). Preclinical studies demonstrate that exoIL-12, an engineered exosome that displays fully active interleukin-12, achieves pronounced tumor retention (roughly a 10-fold increase in intratumoral exposure over recombinant IL-12), drives sustained local IFN-γ production for up to 48 h, and elicits no detectable systemic cytokine release in mice and non-human primates; these pharmacological advantages underpin a forthcoming first-in-human dose-escalation trial in solid tumors (*21*), while exoASO-STAT6 is being evaluated for hepatocellular and colorectal cancers as the first exosomal antisense platform to re-educate tumor-associated macrophages to an M1 phenotype (*22*). Crucially, these precision vesicles dovetail with advances in exosome-based liquid biopsy: circulating exosomal PD-L1 and multi-omic EV panels can now be quantified in microliter plasma samples, enabling ultra-early disease detection, real-time pharmacodynamic monitoring, and rapid identification of resistance pathways (*23,24*). By integrating timely, minimally invasive diagnostics with targeted exosomal payloads, oncology is moving towards a closed-loop paradigm in which early interception, individualized dosing, and adaptive response assessment converge to maximize therapeutic benefit while minimizing collateral toxicity.

Standardization now constitutes the principal bottleneck. The International Society for Extracellular Vesicles published MISEV2023, which lists essential controls for purity, quantification, and function, and yet many diagnostic studies still omit basic purity controls, complicating inter-laboratory comparisons and regulatory review (*25*). Regulatory agencies are debating whether vesicle diagnostics should remain laboratory-developed tests or migrate to full *in vitro* diagnostic oversight, a decision that will shape timelines and post-marketing obligations (*26*). A recent Markov decision analysis in *Gastroenterology* indicated that a triennial blood-based screening test that merely meets the Centers for Medicare & Medicaid Services minimum analytic threshold (sensitivity of 74% and specificity of 90% for colorectal cancer) is overshadowed by annual fecal immunochemical testing (FIT) — that is, it yields fewer quality-adjusted life-years while incurring higher costs, irrespective of how low the per-test price is set. In contrast, modelling shows that a higher-performance assay combining a sensitivity for cancer of at least 90% with a sensitivity for advanced precancerous lesions of 70–80% while maintaining a specificity of 90% could be cost-competitive with FIT, provided its price falls to approximately US $120–140 per test (*27*). Recent state-of-the-field analyses indicated that exosome-oriented technologies — including liquid biopsy platforms — are poised for commercial growth: a pharmaceutical review cited "significant market growth projections" for exosome therapy and reported that the Vesiclepedia database has more than doubled its extracellular vesicle entries since 2019, with 204 exosome-related clinical trials currently registered, collectively signaling rising academic and industrial investment (*28*). Key obstacles remain. Tissue of origin assignment is difficult when multiple organs shed vesicles and machine learning-based deconvolution is only partly effective (*29*). Biological noise from platelet or stromal vesicles increases during infection, trauma, or even vigorous exercise, and threshold definitions that distinguish malignant changes from inflammation are still evolving. Functional validation will clarify whether vesicle markers merely track disease or partly drive progression, a question relevant to eventual therapeutic targeting (*30,31*).

Surface marker capture with antibodies against putative tissue antigens such as L1CAM for the brain, EPCAM for the epithelium, or ASGR1 for the liver is still the most familiar route to enriching vesicles, and yet even carefully optimized protocols show that only about one-half of the immunopurified particles display coherent neuronal co-markers; the yield drops sharply in inflammatory or elderly cohorts, illustrating how marker promiscuity and proteolytic shedding limit specificity (*32,33*). To move beyond single-epitope bias, multiplex single-particle proteomics platforms such as the proximity barcoding assay now read hundreds of surface proteins on individual vesicles and cluster them by origin, but the workflow demands bespoke DNA-antibody libraries, deep sequencing, and days of computation, while still sampling far fewer than a millionth of the vesicles present in a milliliter of plasma (*34*). Bulk RNA approaches instead try to infer provenance computationally: a recent head-to-head comparison of 11 deconvolution algorithms showed that DWLS and CIBERSORTx best explain EV mixtures, and yet even under ideal cell-line conditions they recover only about half of the true variance, and accuracy collapses once platelet and leukocyte vesicles dominate the background (*35,36*). Droplet-level analyses such as SEVtras mine single-cell RNA-seq to score secretion activity directly, offering a clever orthogonal read-out, but performance is tied to the completeness of the underlying tissue atlas and cannot yet quantify the degree of cross-tissue admixture that characterizes plasma (*37*). Extracellular vesicle DNA provides epigenomic barcodes that mirror copy-number and methylation landscapes of donor nuclei; proof-of-principle methylome maps now exhibit a striking concordance with paired tumors, and yet evDNA yields are orders of magnitude lower than cfDNA and bisulphite chemistry erodes what little material is present, making routine multi-omic integration impractical (*38*). Label-free physical sensors provide a reagent-independent route; in a study by Liu *et al*. (*39*), a capillary-phase liquid SERS platform paired with a Bayesian-optimized support vector machine correctly classified plasma extracellular vesicles from stage I–II lung cancer patients versus healthy donors

with an accuracy of 91.5% (95.4% when a convolutional neural network was applied). Although the investigators mitigated matrix artefacts through rigorous baseline correction and by acquiring each spectrum from a fresh aliquot, they still noted that residual lipoproteins can obscure vesicle Raman fingerprints and emphasized the need for validation in larger, more heterogeneous cohorts. Reproducibility also depends on maintaining uniform gold-nanoparticle substrates, the enhancement factors of which can vary between production batches, and on expanding well-annotated spectral libraries for robust machine-learning training. These practical issues — rather than the core sensing principle — remain the principal hurdles to clinical translation of capillary-phase SERS-EV diagnostics. Collectively these findings show that no single modality can yet deliver an absolute, quantitative evaluation of vesicle provenance once multiple organs are shedding vesicles simultaneously; progress will hinge on harmonizing multi-omic reference atlases, standardizing isolation benchmarks across laboratories, and building probabilistic frameworks that integrate orthogonal surface, RNA, lipid and methylation signatures into a unified call for each individual particle.

Exosome research is transitioning from proof of concept experiments to demonstrations of clinical utility. These vesicles combine high abundance, diverse molecular cargo, and direct biological relevance in a single analyte, while recent advances in microfluidic and nanotechnology platforms now enable their routine isolation and analysis. The next critical steps are widespread adoption of MISEV compliant protocols in multicenter trials, establishment of transparent regulatory pathways, and pricing strategies that satisfy health economic models. Achieving these goals could advance cancer diagnosis by months and shift oncology from late stage salvage to true early interception medicine.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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