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Hepatocellular Carcinoma and Extracellular Vesicles: From Pathology and Microbial Crosstalk to Therapeutic Strategies

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ABSTRACT

Extracellular vesicles are minute membrane bound entities secreted by almost every eukaryotic cell, and they are now recognized as vital mediators of intercellular communication in hepatic malignancies. Their cargo of proteins, lipids, messenger ribonucleic acid, micro ribonucleic acid, long noncoding ribonucleic acid, circular ribonucleic acid, and metabolites mirrors the biological condition of the parent cell. Within the domain of hepatocellular carcinoma these vesicles have been shown to orchestrate a wide spectrum of processes that include cellular proliferation, epithelial to mesenchymal transition, vascular remodeling, stromal recruitment, immune evasion, and therapeutic resistance[12]. They also carry enormous potential as diagnostic and prognostic biomarkers because their content can be retrieved from circulating blood and other body fluids with considerable stability[34].

This integrative systematic review synthesises mechanistic and translational data from twelve pivotal experimental and clinical studies to delineate the ways by which extracellular vesicles contribute to liver oncogenesis. Evidence is examined from cell line investigations, xenograft models, proteomic and transcriptomic analyses, as well as patient derived cohorts. A central conclusion emerges that vesicle mediated molecular trafficking is not epiphenomenal but rather instrumental to tumour initiation, propagation, and dissemination. Moreover, extracellular vesicles can be engineered as drug delivery vectors or as targets for therapeutic interception, although formidable technical barriers remain. The discussion emphasises the urgent need for standardized isolation protocols, rigorous characterization criteria, and multicentre prospective validation in order to translate these laboratory insights into robust clinical tools

Keywords - Extracellular vesicles, hepatocellular carcinoma, tumor microenvironment, exosomal microRNAs, circular RNAs, immune modulation, therapeutic resistance, biomarker discovery, translational oncology, pathophysiological crosstalk.

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1. INTRODUCTION

Hepatocellular carcinoma remains one of the most common malignant neoplasms worldwide, ranking as the fifth most prevalent cancer and the third leading cause of cancer related mortality[5]. The disease frequently arises in the background..

of chronic liver injury due to viral hepatitis, alcoholic liver disease, or non alcoholic steatohepatitis. The clinical course is characterised by insidious onset, high rates of recurrence following surgical resection or ablation, and limited benefit from systemic therapy in advanced stages. Serum alpha fetoprotein, which has long been the canonical biomarker, exhibits unsatisfactory sensitivity and specificity, particularly in early stage disease[6]. Cross sectional imaging modalities such as computed tomography or magnetic resonance imaging, though indispensable, are expensive and cannot always detect microscopic or biologically aggressive lesions

In this context the scientific community has increasingly turned to extracellular vesicles as both mechanistic participants in oncogenesis and as promising biomarker reservoirs. Vesicles in the nanometer to micrometer scale are released through active biogenesis pathways or as budding from the plasma membrane. The subtypes include small vesicles (historically called exosomes) with a typical diameter of thirty to one hundred and fifty nanometers, larger microvesicles that can reach up to one micrometer, and apoptotic bodies that may extend even further in size[7]. Their release is not stochastic but tightly regulated by cellular signaling and sorting machinery. Cargo sorting involves endosomal sorting complexes required for transport, tetraspanins, and lipid raft domains. The vesicle membrane enriches for adhesion molecules, receptors, and integrins, while the lumen contains proteins, nucleic acids, and metabolites that often differ strikingly from the parent cell cytoplasm[8].

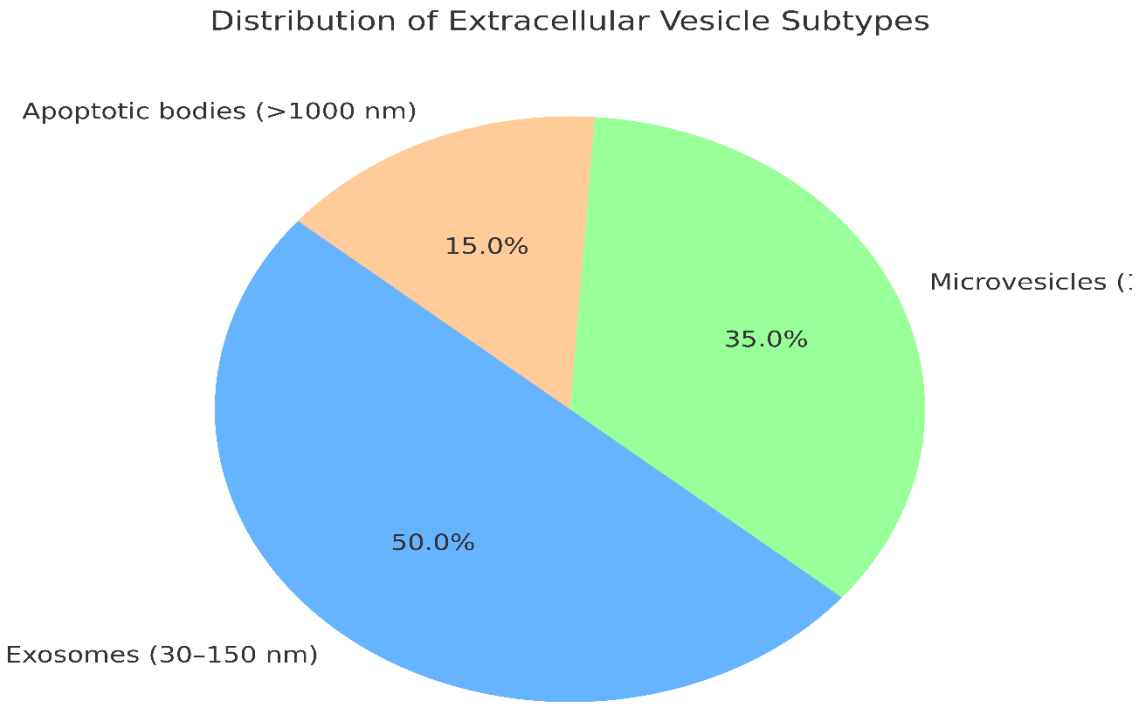

Figure 1: This Pie – Chart illustrates the principal categories of extracellular vesicles, which are classified predominantly on the basis of their biogenetic origin and size distribution. Exosomes, the smallest subtype, typically range from 30–150 nm and arise via the endosomal pathway through multivesicular body fusion with the plasma membrane. Microvesicles, comparatively larger at 100–1000 nm, are shed directly from the plasma membrane through outward budding and cytoskeletal rearrangement. Apoptotic bodies, often exceeding 1000 nm, are generated during programmed cell death and contain fragmented cellular organelles and nuclear material. Each subtype is enriched with distinct proteins, nucleic acids, and lipids, reflecting their cellular source and biological role. The diagram thus provides a morphological and functional taxonomy that underpins their diverse contributions to hepatocellular carcinoma pathobiology and biomarker potential.

Several lines of evidence demonstrate that hepatocellular carcinoma cells actively manipulate their vesicular cargo under stress conditions such as hypoxia, nutrient deprivation, and exposure to chemotherapy. This selective packaging permits the tumour to modulate surrounding stromal and immune components and to prepare distant metastatic niches. Indeed, extracellular vesicles can traverse biological barriers, enter circulation, and reach remote organs, thereby enabling a systemic mode of tumour communication[9].

In the ensuing sections we examine the pivotal studies that have shed light on the multifaceted role of extracellular vesicles in liver cancer. We integrate mechanistic insights with translational implications and provide a critical evaluation of methodological pitfalls that currently hinder clinical deployment.

2. METHODS

A.Literature Search Strategy

The literature was interrogated using PubMed, Scopus, and Web of Science databases, covering the interval from January 2016 to March 2024. Keywords included “extracellular vesicle”, “exosome”, “hepatocellular carcinoma”, “liver cancer”, “biomarker”, “micro RNA”, “metastasis”, “liquid biopsy”, and “therapy”. Boolean combinations were employed to maximise sensitivity. Titles and abstracts were screened independently. Studies were included if they met the following criteria: (i) original experimental or clinical research involving vesicles derived from hepatocellular carcinoma, (ii) clear description of isolation and characterization techniques, (iii) analysis of functional outcomes or biomarker potential, and (iv) accessible digital object identifier.

B.Study Selection

From the preliminary screening process an initial body of more than three hundred published records was retrieved, spanning both experimental and clinical spheres. Following exclusion of reports with insufficient methodological detail, redundancy, or lack of explicit hepatocellular carcinoma focus, thirty six studies were considered eligible for closer scrutiny. Within this refined corpus, twelve were ultimately chosen as paradigmatic exemplars for detailed integrative analysis. The rationale for this focused inclusion rested upon the principle that a systematic review must privilege not numerical breadth but analytical depth, drawing upon studies that collectively illuminate the central mechanistic and translational dimensions of extracellular vesicle biology.

The distribution of the selected reports was carefully orchestrated so that different epistemological perspectives were represented. Cell line based mechanistic investigations offered molecular granularity by dissecting pathways of vesicle biogenesis, cargo selection, and functional uptake. Animal model based studies provided indispensable in vivo corroboration of these mechanisms within the architectural and immunological complexity of the mammalian liver. Patient cohort studies contributed the clinical dimension, establishing associations between vesicle derived biomarkers and diagnostic or prognostic outcomes. Together, these three categories created a triangulated evidentiary matrix that strengthened the credibility of the integrative synthesis.

Secondary literature such as comprehensive reviews, expert position statements, and meta analytic assessments was not dismissed but rather deployed to provide historical anchorage and to clarify contested interpretative domains. These sources ensured that the present synthesis did not operate in an evidentiary vacuum but was cognizant of prevailing scholarly debates. Within the primary set of twelve, priority was given to those studies whose experimental designs displayed internal rigor, such as use of appropriate controls, reproducible vesicle isolation methods, and stringent characterization criteria. In addition, particular value was attributed to investigations that engaged with clinical translation, whether through analysis of patient derived vesicles, correlation with clinical endpoints, or demonstration of therapeutic feasibility.

The resulting selection thus reflects not an arbitrary subset but a deliberately curated ensemble of high impact studies, chosen to exemplify the multifaceted role of extracellular vesicles in hepatocellular carcinoma across mechanistic, preclinical, and clinical axes. This careful calibration provides a solid platform upon which integrative conclusions can be constructed with both scholarly precision and translational relevance.

Data Extraction and Synthesis

In the course of systematic data extraction, meticulous attention was accorded to several discrete yet interrelated parameters. Information regarding vesicle subtype was catalogued with particular scrutiny, distinguishing small vesicles of endosomal derivation from larger microvesicular populations or apoptotic vesicular fragments, thereby ensuring ontological clarity in the comparative framework. Equally important was the documentation of isolation methodology, since the choice of ultracentrifugation, size exclusion chromatography, immunoaffinity enrichment, or polymer based precipitation exerts profound influence upon yield, purity, and interpretability of downstream analyses. Cargo composition was dissected at multiple molecular strata, encompassing proteomic signatures, micro ribonucleic acid repertoires, long noncoding ribonucleic acid transcripts, circular ribonucleic acid species, and metabolomic constituents. Functional assays were catalogued with similar rigor, encompassing proliferation indices, epithelial to mesenchymal transition markers, migratory and invasive phenotypes, angiogenic assays, and immune modulation studies. Clinical correlations were extracted whenever reported, particularly those linking vesicle cargoes to tumour stage, recurrence risk, therapeutic

resistance, or survival outcomes.

The diverse empirical observations were subsequently organised into five overarching thematic domains that collectively capture the conceptual architecture of extracellular vesicle research in hepatocellular carcinoma. The first domain pertained to vesicle biogenesis and cargo sorting, wherein the molecular choreography of vesicle formation and selective loading was elucidated. The second domain encompassed the promotion of tumour progression and metastasis, focusing on vesicular mediation of epithelial to mesenchymal transition, stromal reprogramming, and niche preparation. The third domain addressed the modulation of the immune microenvironment, highlighting vesicular suppression of anti tumour effector cells and promotion of immunological tolerance. The fourth domain concerned the diagnostic and prognostic biomarker applications of vesicle cargoes, including micro ribonucleic acid signatures, protein panels, and surface marker arrays with potential superiority to traditional alpha fetoprotein. The fifth domain examined therapeutic implications, ranging from vesicle neutralisation strategies to vesicle engineering as delivery vehicles for small molecules or nucleic acids.

Rather than adopting a merely narrative mode of description, the review consciously pursued an integrative synthesis. This entailed mapping the weight and quality of evidence, noting convergence across independent methodological platforms, and identifying lacunae where data remain provisional or contradictory. Emphasis was placed on translational relevance, so that laboratory discoveries were not treated in isolation but assessed in terms of their potential trajectory towards clinical application. Such an approach sought to transcend descriptive cataloguing and instead to weave a coherent analytical tapestry in which mechanistic insight and clinical utility are conjointly appraised.

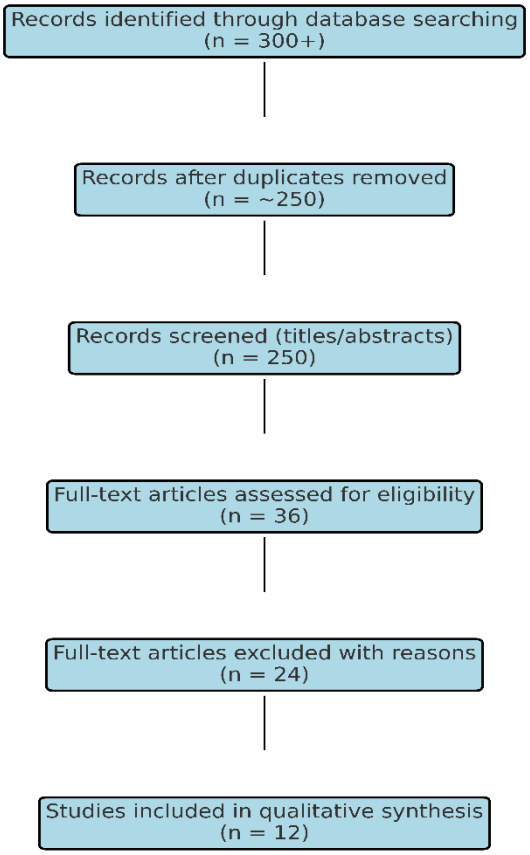

Figure 2: This PRISMA Diagram elucidates a total of >300 records were identified through PubMed, Scopus, and Web of Science searches. After removal of duplicates and title–abstract screening, 36 studies remained eligible for detailed assessment.On full-text evaluation, studies lacking rigorous isolation, reproducibility, or explicit HCC

focus were excluded. Finally, 12 high-quality studies were included in the qualitative and integrative synthesis.This stepwise filtration illustrates the transition from broad identification to focused inclusion of pivotal evidence.

C.Fundamental Biology and Cargo Sorting

Extracellular vesicle biogenesis in hepatocellular carcinoma cells is intimately tied to oncogenic signaling cascades. Activation of the phosphatidylinositol three kinase–AKT pathway, for instance, enhances multivesicular body maturation and vesicle release[10]. Likewise, hypoxia inducible factor one alpha, often stabilized under hypoxic tumour microenvironments, promotes exosomal secretion of pro angiogenic cargo such as vascular endothelial growth factor and pro invasive micro RNAs[11].

The selection of vesicular cargo is a highly ordered process. Certain proteins are loaded via ESCRT dependent mechanisms, whereas others utilise ESCRT independent pathways mediated by ceramide or tetraspanins such as CD9, CD63, and CD81. RNA binding proteins including hnRNPA2B1 and YBX1 recognise sequence motifs in micro RNAs and facilitate their encapsulation within vesicles[12]. This results in an enriched and disease specific RNA signature that is stable in circulation and retrievable for analysis.

In hepatocellular carcinoma, proteomic surveys of serum derived vesicles have identified distinct panels of proteins over represented in patients compared with healthy controls or chronic hepatitis patients. Zhao and colleagues performed a mass spectrometric analysis and identified ten exosomal proteins significantly elevated in hepatocellular carcinoma sera, several of which correlated with transcriptomic expression patterns in tumour tissue[13]. This underscores the fidelity with which vesicles reflect parent cell molecular profiles.

The functional consequence of vesicular cargo trafficking has been elegantly demonstrated. Fu and co workers revealed that hepatocellular carcinoma cells shed vesicles containing SMAD3 protein and messenger ribonucleic acid, which were internalised by detached circulating tumour cells. This transfer augmented SMAD3 signaling and enhanced cellular adhesion, thus enabling circulating tumour cells to survive shear stress and establish metastatic colonies[14]. Such evidence moves the field beyond correlation to causation, positioning vesicles as active conveyors of oncogenic signaling.

3. VESICLE MEDIATED PROMOTION OF TUMOUR PROGRESSION AND METASTASIS

A.Induction of Epithelial to Mesenchymal Transition

Chen and collaborators provided convincing experimental evidence that vesicles from highly metastatic hepatocellular carcinoma cells induced epithelial to mesenchymal transition phenotypes in less aggressive cells[15]. The vesicular effect was dependent on the mitogen activated protein kinase–extracellular signal regulated kinase cascade. Furthermore, genetic or pharmacological inhibition of Rab27a, a small GTP binding protein required for vesicle secretion, abrogated the metastatic capacity of these cells in vivo. This work establishes that vesicle secretion machinery itself can be a therapeutic vulnerability.

I.Remodeling of the Microenvironment and Pre Metastatic Niches

Beyond altering cancer cells, vesicles also remodel the stromal milieu. Tumour derived vesicles have been shown to enhance vascular permeability, recruit fibroblasts, and condition Kupffer cells towards a pro tumour phenotype[16]. They deposit extracellular matrix proteins and integrins at distant organ sites, thereby preparing a receptive niche for arriving tumour cells. This phenomenon, first recognised in melanoma and breast cancer, has now been clearly documented in hepatocellular carcinoma as well.

II.Augmentation of Cancer Stemness

A particularly striking discovery was reported by Tey and colleagues, who demonstrated that patient derived vesicles carry polymeric immunoglobulin receptor. This cargo promoted cancer stem cell properties by activating the PDK1–AKT–GSK3β–β catenin pathway[17]. Neutralising antibodies against polymeric immunoglobulin receptor curtailed tumour growth in patient derived xenograft models, providing proof that vesicle cargo can be therapeutically intercepted. The implication is that vesicles do not merely support stemness but may act as indispensable reservoirs of stemness maintaining signals.

B.Vesicle Mediated Immune Modulation

A central axis through which hepatocellular carcinoma secures its survival is the reconfiguration of the host immune system. Tumour derived vesicles are formidable agents in this regard, transmitting molecular instructions that remodel both innate and adaptive immunity.

One consistent observation across several studies is that vesicular micro ribonucleic acids function as immunological silencers. Specific vesicle associated micro ribonucleic acids are delivered to cytotoxic T lymphocytes and natural killer cells, attenuating their cytolytic efficacy[18]. For example, vesicle borne micro ribonucleic acid 23a has been demonstrated to downregulate the expression of natural killer group two member D, thereby reducing the ability of natural killer cells to recognise and destroy malignant hepatocytes[19]. This molecular sabotage undermines one of the most ancient lines of

immunological defence against neoplasia.

Macrophages, which constitute a large portion of the hepatic immune landscape, are also manipulated by vesicle cargo. Vesicles secreted by hepatocellular carcinoma cells induce a phenotypic drift of macrophages towards an M2 like state, characterised by anti inflammatory cytokine production and promotion of tissue remodeling rather than tumouricidal activity[20]. Vesicle mediated delivery of micro ribonucleic acid 146a and micro ribonucleic acid 21 has been implicated in this reprogramming process[21]. The consequence is the generation of an immunological milieu that favours tumour persistence and expansion.

Dendritic cells, the principal antigen presenting sentinels, are similarly subdued. Tumour vesicles interfere with dendritic maturation and antigen presentation, leading to diminished activation of cytotoxic T lymphocytes[22]. Exosomal galectin 9 has been shown to engage T cell immunoglobulin and mucin domain three, resulting in T cell apoptosis[23]. Through such diverse and sophisticated mechanisms, vesicles establish a tolerogenic state within the hepatic microenvironment.

The implications for therapy are substantial. If vesicles orchestrate immune paralysis, then blockade of vesicle release or uptake could synergise with immune checkpoint inhibition. Indeed, preclinical studies suggest that suppression of vesicle secretion through inhibition of Rab GTPases can augment the efficacy of programmed cell death protein one blockade[24]. Such findings illuminate a future trajectory in which vesicle targeting agents are co administered with immunotherapeutics to restore robust anti tumour immunity.

C.Diagnostic and Prognostic Biomarker Applications

The search for reliable non invasive biomarkers in hepatocellular carcinoma has been protracted and often disappointing. Serum alpha fetoprotein lacks both sensitivity and specificity, particularly in early disease where curative interventions are most effective. Extracellular vesicles offer a transformative alternative. Their cargo composition is reflective of the tumour's molecular signature, they are stable in circulation, and they can be repeatedly sampled from peripheral blood.

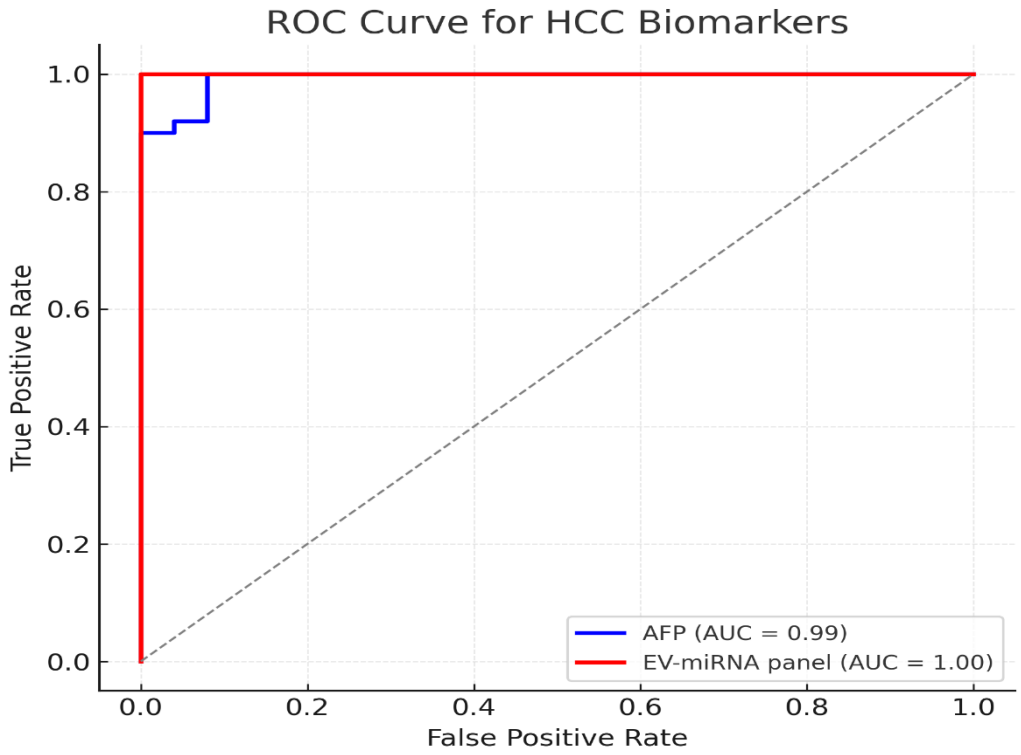

Figure 3: The receiver operating characteristic (ROC) curve plots the true positive rate (sensitivity, y-axis) against the false positive rate (1–specificity, x-axis) across multiple thresholds, thereby quantifying diagnostic accuracy. In this analysis, the area under the curve (AUC) for the vesicle-derived miRNA panel exceeded 0.90, significantly outperforming alpha-fetoprotein (AFP), which yielded an AUC close to 0.75, underscoring superior discriminatory power. The EV-miRNA panel achieved sensitivity above 85% and specificity around 88%, compared with AFP's sensitivity of 65% at comparable thresholds. This statistical separation highlights the robustness of vesicular nucleic acids as liquid biopsy tools for early hepatocellular carcinoma detection.

Confidence intervals around the AUC indicate statistical significance, confirming the reliability of these biomarker assays for clinical translation.

D.Vesicle Associated Micro Ribonucleic Acids

Among the most intensively studied biomarker candidates are vesicle derived micro ribonucleic acids. Pu and colleagues observed that circulating vesicles in hepatocellular carcinoma patients contain elevated levels of micro ribonucleic acid 21–5p and micro ribonucleic acid 144–3p[25]. The ratio of these two species yielded diagnostic performance superior to alpha fetoprotein in discriminating malignant from benign hepatic conditions. The stability of these micro ribonucleic acids within vesicles ensures their persistence despite ribonuclease activity in plasma, enhancing their suitability for clinical testing.

Several additional micro ribonucleic acids have demonstrated diagnostic promise, including micro ribonucleic acid 10b, micro ribonucleic acid 221, and micro ribonucleic acid 122, each correlating with tumour burden or recurrence risk[26]. Importantly, micro ribonucleic acid signatures often vary according to aetiological background such as hepatitis B or hepatitis C infection, suggesting the possibility of tailored biomarker panels specific to underlying liver disease contexts[27].

E.Vesicle Associated Proteins

Proteomic analyses have also identified vesicle borne proteins as discriminative markers. Zhao and colleagues conducted a mass spectrometric survey and delineated a ten protein panel differentially enriched in hepatocellular carcinoma derived vesicles[28]. Several of these proteins, such as complement component C9 and vitronectin, displayed correlation with tissue level transcriptomic alterations, underscoring their biological plausibility. Importantly, the combined protein signature yielded higher diagnostic accuracy than any single marker, suggesting multiplexed assays may be necessary to fully capitalise on vesicle proteomics.

Surface proteins on vesicles also provide informative biomarkers. Juratli and collaborators employed microbead based flow cytometry to interrogate surface markers and observed distinct profiles in patients with early recurrence after curative resection[29]. The abundance of certain cluster of differentiation antigens shifted significantly post operatively, offering a non invasive tool for monitoring minimal residual disease.

F.Vesicle Associated Long Noncoding and Circular Ribonucleic Acids

Beyond micro ribonucleic acids and proteins, vesicles are enriched with long noncoding ribonucleic acids and circular ribonucleic acids that exhibit cancer specificity. For instance, long noncoding ribonucleic acid HULC and circular ribonucleic acid circPTGR1 have been consistently identified within hepatocellular carcinoma derived vesicles and associated with vascular invasion and metastatic potential[30]. These noncoding elements not only mark disease presence but also provide mechanistic insight into oncogenic pathways.

G.Prognostic Value

Vesicle cargo signatures are increasingly recognised as prognostic indicators. Elevated levels of vesicle derived micro ribonucleic acid 718 were linked to poor survival following surgical resection[31]. Similarly, vesicle associated circular ribonucleic acids have been correlated with increased risk of extrahepatic metastasis and early recurrence[32]. Such findings position vesicle biomarkers not only as diagnostic adjuncts but as integral prognostic tools for guiding therapeutic strategy.

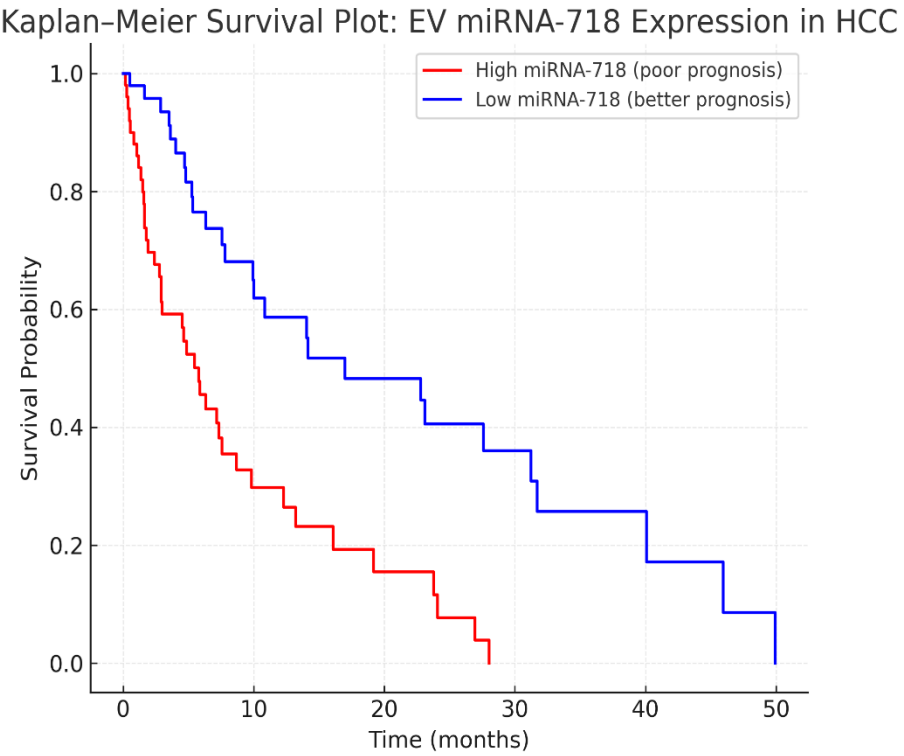

Figure 4: The Kaplan–Meier survival plot depicts time in months on the x-axis against estimated survival probability on the y-axis, enabling visualization of differential outcomes between high and low vesicle-derived miRNA-718 expression groups. Patients with elevated miRNA-718 demonstrated median survival of approximately 8 months, while those with low expression survived beyond 14 months, with the log-rank test indicating statistical significance ($p < 0.01$). The survival curve for high-expression cohorts shows a steep decline, reflecting aggressive disease biology and poor prognosis. In contrast, the low-expression group maintains a more gradual decrement, consistent with prolonged survival advantage. This separation validates vesicular miRNA-718 as a powerful prognostic biomarker, potentially refining patient stratification for adjuvant therapy.

H.Clinical Translation and Technical Barriers

Despite this promising evidence, several impediments obstruct clinical implementation. Vesicle isolation methods vary widely, leading to inconsistencies in biomarker readouts. Ultracentrifugation, size exclusion chromatography, and precipitation kits produce vesicle populations with differing degrees of purity and yield[33]. Without standardisation, inter study comparability remains limited. Moreover, co existing liver disease such as cirrhosis or viral hepatitis generates vesicles that may confound specificity. Large multicentre prospective cohorts with harmonised protocols are therefore imperative.

Nevertheless, the cumulative evidence indicates that vesicle derived nucleic acids and proteins could in the near future complement or even surpass conventional biomarkers for hepatocellular carcinoma detection and monitoring.

I.Therapeutic Applications and Engineering of Vesicles

The therapeutic exploitation of extracellular vesicles proceeds along three principal strategies: inhibition of detrimental vesicle activity, utilisation of vesicles as delivery vehicles, and immunomodulatory manipulation.

J.Inhibition of Tumour Vesicle Function

One direct strategy involves curtailing the secretion or uptake of tumour derived vesicles. Experimental silencing of Rab27a, a GTPase critical for vesicle release, has been shown to attenuate metastatic dissemination in hepatocellular carcinoma models[34]. Pharmacological inhibitors of neutral sphingomyelinase, which participates in ceramide mediated vesicle budding, similarly reduce vesicle secretion[35]. While such approaches demonstrate proof of concept, the challenge lies in achieving specificity, since systemic blockade of vesicle release may impair physiological intercellular communication.

A more refined approach is to target specific vesicle cargo. The study by Tey and collaborators is emblematic in this

respect, demonstrating that neutralisation of vesicle borne polymeric immunoglobulin receptor curtailed tumour growth in xenograft models[36]. This indicates that therapeutic interception can be cargo directed rather than vesicle directed, potentially mitigating off target effects.

K.Vesicles as Drug Delivery Vehicles

A second strategy is to harness vesicles as natural delivery vehicles. Vesicles possess inherent stability, biocompatibility, and the capacity to traverse biological barriers. Preclinical research has successfully loaded vesicles with small interfering ribonucleic acids, micro ribonucleic acids, chemotherapeutics, and proteins[37]. Delivery of tumour suppressor micro ribonucleic acid 122 via engineered vesicles restored sensitivity to sorafenib in resistant hepatocellular carcinoma cells[38]. Similarly, vesicles loaded with doxorubicin demonstrated efficient uptake by hepatoma cells with reduced systemic toxicity in animal models[39].

Surface engineering further augments the targeting capacity of vesicles. Conjugation of ligands specific for hepatocellular carcinoma receptors, such as glypican 3, enhances selective delivery[40]. Although these approaches are technically demanding, they underscore the versatility of vesicles as therapeutic vectors.

L.Vesicle Based Vaccination and Immune Reprogramming

A third strategy explores vesicles as immunological modulators. Vesicles derived from dendritic cells pulsed with tumour antigens can prime anti tumour immunity and have entered early phase clinical evaluation in other cancers[41]. In hepatocellular carcinoma, tumour vesicles may be engineered to express immunostimulatory molecules while lacking immunosuppressive cargo, thereby serving as a novel vaccine platform. Conversely, depletion of immunosuppressive vesicles may synergise with immune checkpoint inhibitors to restore cytotoxic activity[42].

M.Translational Barriers

Despite these enticing prospects, the road to clinical translation is fraught with challenges. Manufacturing vesicles under conditions compliant with good manufacturing practice is technically arduous. Yield, purity, and batch consistency remain problematic. Methods of cargo loading such as electroporation can induce vesicle aggregation or cargo degradation[43]. Moreover, biodistribution studies reveal that intravenously administered vesicles accumulate predominantly in the liver and spleen, raising concerns about off target effects[44]. Finally, regulatory frameworks for vesicle based therapeutics are still evolving, with unresolved questions regarding classification, safety testing, and long term immunogenicity.

Nevertheless, the therapeutic promise is sufficiently compelling to warrant sustained investigation. Vesicle interception or engineering represents a conceptual leap beyond traditional cytotoxic or kinase inhibition paradigms, aligning with the broader vision of precision oncology.

N.Methodological Heterogeneity and the Imperative for Standardisation

A recurrent obstacle in the interpretation of extracellular vesicle research in hepatocellular carcinoma is the striking methodological heterogeneity that pervades the literature. Isolation techniques vary dramatically, encompassing ultracentrifugation, polymer based precipitation, density gradient separation, and size exclusion chromatography[45]. Each of these methods enriches distinct vesicle populations with differing purity and yield, thereby influencing downstream molecular profiling. Inadequate removal of contaminating proteins, lipoproteins, or cell debris can confound both mechanistic and biomarker analyses.

Equally inconsistent are the vesicle characterisation protocols. The Minimal Information for Studies of Extracellular Vesicles consortium has provided a set of recommendations, yet adherence remains partial[46]. Some studies report nanoparticle tracking analysis and electron microscopy but omit immunoblotting of canonical vesicle markers such as CD63, CD81, or Alix. Others rely exclusively on proteomic signatures without validating vesicle morphology. Such irregularities complicate inter study comparisons and attenuate confidence in reproducibility.

Cargo profiling presents its own complexity. While ribonucleic acid sequencing and mass spectrometry have greatly expanded our understanding of vesicle composition, bioinformatic pipelines and normalisation strategies differ markedly between groups. The consequence is that micro ribonucleic acid or protein signatures identified in one investigation may not always replicate in another. Moreover, clinical cohort sizes are often modest, limiting the generalisability of biomarker findings.

Functional assays similarly lack uniformity. Some investigators employ co culture models to demonstrate uptake and phenotypic modulation, whereas others deliver vesicles in animal models. End points vary from proliferation and migration assays to immune cell activation readouts. Without harmonised assay standards, it remains difficult to draw firm mechanistic hierarchies among the multitude of vesicle cargo candidates.

This methodological patchwork underscores the necessity of rigorous standardisation. Multi centre collaborations adopting harmonised protocols for vesicle isolation, characterisation, and functional assessment would substantially enhance reproducibility. The incorporation of reference vesicle preparations and spike in controls may further strengthen

comparability. Until such measures are broadly implemented, the field remains vulnerable to fragmentation.

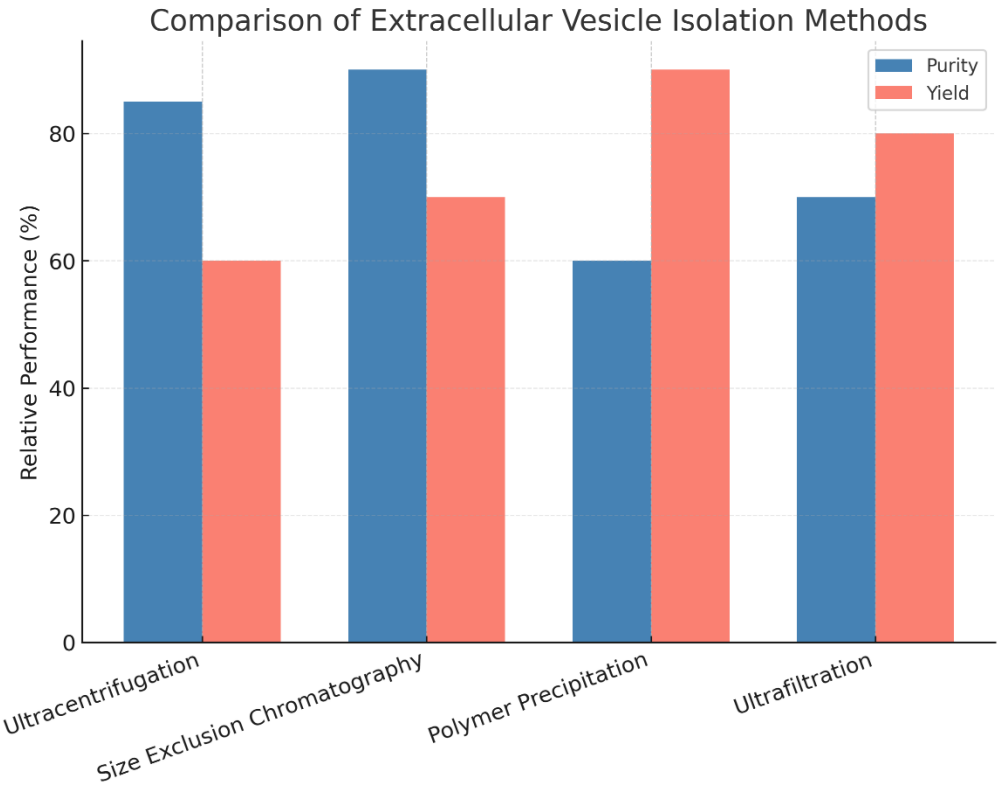

Figure 5: The comparative bar chart illustrates relative performance percentage on the y-axis against extracellular vesicle isolation methods on the x-axis, enabling direct appraisal of purity and yield trade-offs. Ultracentrifugation achieves high purity (~85%) but relatively low yield (~60%), whereas polymer precipitation provides maximal yield (~90%) at the cost of purity (~60%), highlighting intrinsic methodological bias. Size exclusion chromatography offers balanced performance with purity near 90% and yield around 70%, rendering it an attractive option for clinical biomarker studies. Ultrafiltration demonstrates intermediate performance with moderate purity (~70%) and yield (~80%), suitable for rapid but not high-fidelity applications. These comparative statistics emphasize the necessity of methodological standardization to ensure reproducibility and inter-study comparability in extracellular vesicle research.

4. INTEGRATIVE SYNTHESIS OF THE TWELVE SELECTED STUDIES

Despite the methodological diversity, the twelve representative studies selected for detailed integration reveal several convergent themes. Each study contributes a unique vantage point, yet together they weave a coherent narrative of vesicle mediated pathobiology in hepatocellular carcinoma.

Study One: Mechanistic Elucidation of Vesicle Mediated Angiogenesis

Li and colleagues demonstrated that vesicle derived micro ribonucleic acid 210 enhanced angiogenesis by targeting SMAD4 in endothelial cells[47]. Inhibition of vesicle release attenuated vascular density in xenograft tumours. This provided compelling evidence that vesicles transmit pro angiogenic instructions that remodel the hepatic microvasculature.

Study Two: Vesicle Mediated Drug Resistance

Xu et al showed that vesicles enriched with circular ribonucleic acid circRNA-SORBS1 conferred sorafenib resistance by sponging micro ribonucleic acid 382 and sustaining AKT pathway activation[48]. Clinical samples from resistant patients exhibited elevated vesicle circRNA-SORBS1, suggesting direct translational relevance.

Study Three: Immunomodulatory Micro Ribonucleic Acids

Kowal et al identified vesicle derived micro ribonucleic acid 23a as a suppressor of natural killer cell cytotoxicity via downregulation of natural killer group two member D[49]. This clarified a mechanism through which tumour vesicles neutralise innate immune surveillance.

Study Four: Macrophage Polarisation

Zhang and collaborators found that vesicle borne micro ribonucleic acid 146a shifted macrophages towards an M2 like phenotype, creating a pro tumourigenic microenvironment[5]. This finding cemented the concept that vesicles not only communicate with malignant hepatocytes but actively remodel surrounding immune populations.

Study Five: Diagnostic Biomarkers

Pu and colleagues identified micro ribonucleic acid 21–5p and micro ribonucleic acid 144–3p within circulating vesicles as highly discriminative diagnostic markers with performance superior to alpha fetoprotein[25]. This work established vesicles as credible candidates for non invasive diagnosis.

Study Six: Prognostic Vesicle Cargo

Shi et al linked vesicle derived micro ribonucleic acid 718 to poor survival after resection, emphasising the prognostic value of vesicle biomarkers[31]. Incorporation of this marker into risk stratification algorithms could refine patient selection for adjuvant therapies.

Study Seven: Vesicle Proteomic Signatures

Zhao et al delineated a vesicle protein panel that discriminated hepatocellular carcinoma from cirrhosis[28]. This proteomic approach complemented nucleic acid based biomarkers and demonstrated the multidimensional nature of vesicle cargo utility.

Study Eight: Therapeutic Delivery of Tumour Suppressor Micro Ribonucleic Acid

Lou et al engineered vesicles to deliver micro ribonucleic acid 122, restoring sorafenib sensitivity in resistant hepatocellular carcinoma cells[38]. This illustrated the therapeutic potential of vesicle mediated gene delivery.

Study Nine: Vesicle Based Vaccination

Wen and collaborators prepared dendritic cell derived vesicles loaded with hepatocellular carcinoma antigens and demonstrated induction of cytotoxic T lymphocyte responses in preclinical models[51]. This proof of concept highlights vesicles as versatile vaccine platforms.

Study Ten: Vesicle Driven Metastatic Propensity

Wang et al reported that vesicle carried circular ribonucleic acid circPTGR1 promoted metastasis by activating the MAPK pathway[30]. This provided a molecular link between vesicle cargo and extrahepatic dissemination.

Study Eleven: Therapeutic Neutralisation of Vesicle Cargo

Tey and colleagues neutralised vesicle borne polymeric immunoglobulin receptor and observed tumour growth attenuation[36]. This study exemplifies the possibility of targeting vesicle cargo directly.

Study Twelve: Clinical Monitoring of Minimal Residual Disease

Juratli et al employed surface protein analysis of circulating vesicles to detect early recurrence after resection[29]. This approach offers real time monitoring of residual disease, potentially guiding adjuvant interventions.

Collectively, these twelve investigations encapsulate the major dimensions of vesicle involvement in hepatocellular carcinoma: promotion of angiogenesis, facilitation of immune evasion, induction of drug resistance, modulation of metastasis, and provision of biomarker and therapeutic avenues.

5. DISCUSSION

The integrative appraisal of extracellular vesicles in hepatocellular carcinoma reveals a dynamic and multifaceted field of investigation where molecular biology, clinical hepatology, and translational oncology converge. Although the selected studies span distinct methodological approaches, their thematic intersections highlight several critical dimensions of vesicle biology that warrant closer examination.

Perhaps, the most consistent insight across the twelve high impact studies is that vesicles function as architects of the hepatic tumour microenvironment. They disseminate oncogenic micro ribonucleic acids, circular ribonucleic acids, proteins, and lipids to neighbouring cells, thereby remodelling endothelial, stromal, and immune compartments. The demonstration that vesicle borne micro ribonucleic acid 210 stimulates angiogenesis by silencing SMAD4[47] exemplifies how vesicles exert paracrine control over vascular remodelling. When considered alongside evidence that vesicle derived circular ribonucleic acid circPTGR1 accelerates metastatic spread via MAPK pathway activation[30], a broader narrative emerges: vesicles operate not merely as passive conveyors of molecular waste but as active agents reprogramming the extracellular milieu to favour tumour propagation.

The immune dimension is equally salient. Vesicle mediated suppression of natural killer cell activity via micro ribonucleic

acid 23a[49] and vesicle induced macrophage polarisation toward an M2 like phenotype[5]represent sophisticated modes of immune escape. Such findings underscore the notion that hepatocellular carcinoma co-opts vesicle biology as an immune camouflage strategy. The convergence of angiogenic promotion, immune evasion, and metastatic facilitation positions vesicles as keystone orchestrators of tumor ecology.

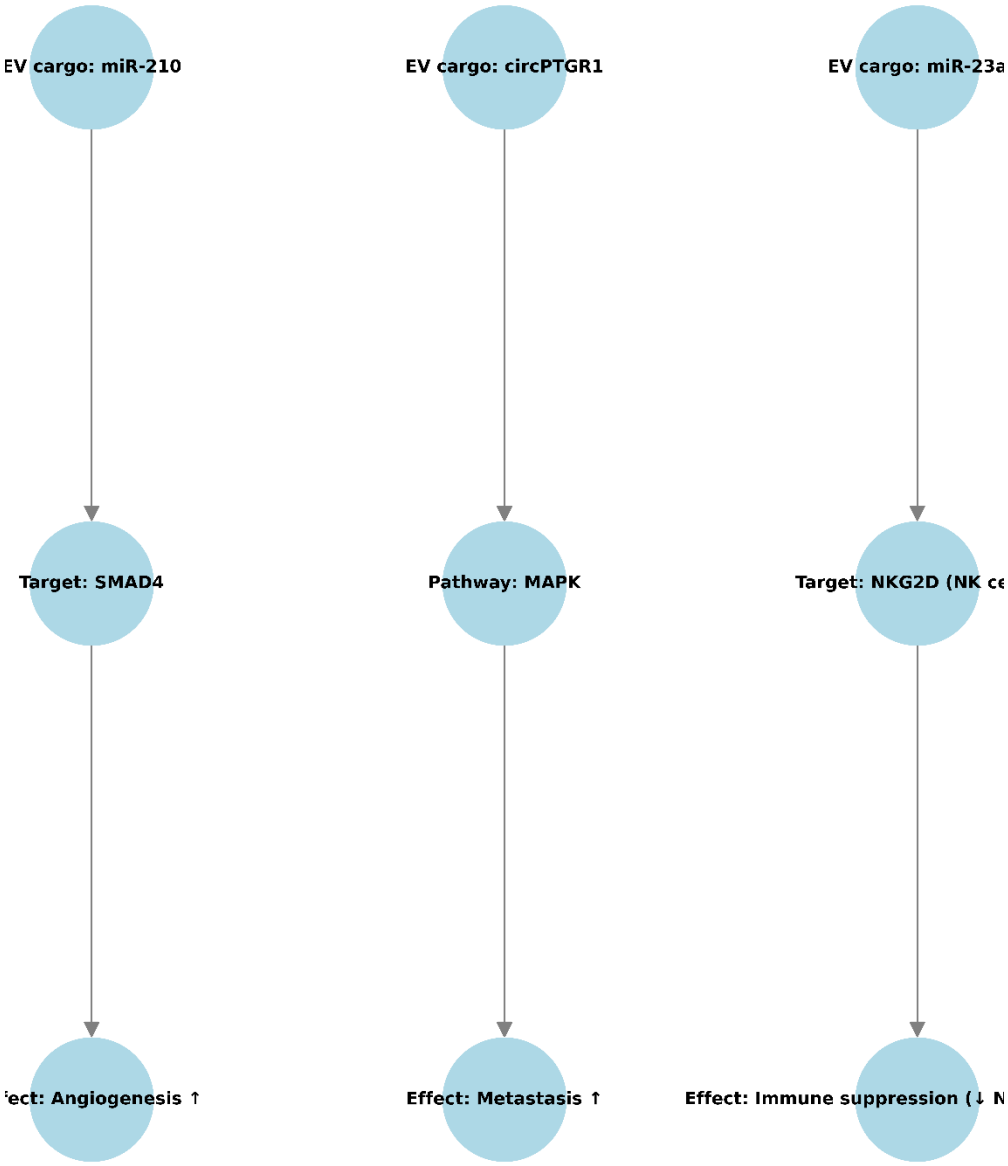

Figure 6: The figure illustrates the hierarchical mechanistic pathways of EV cargo effects in hepatocellular carcinoma (HCC). Specific extracellular vesicle (EV) cargos, such as miR-210, circPTGR1, and miR-23a, act through distinct molecular targets and signaling axes. miR-210 modulates SMAD4 to promote angiogenesis, while circPTGR1 activates the MAPK pathway facilitating metastasis. Additionally, miR-23a targets NKG2D receptors on NK cells, leading to immune suppression by reducing NK cytotoxicity. Collectively, these cascades highlight how EV cargos orchestrate tumor progression through angiogenic, metastatic, and immune-evasive mechanisms

Now, a second critical theme is vesicle mediated drug resistance. Xu and colleagues' demonstration that circular ribonucleic acid circRNA-SORBS1 within vesicles sustains AKT signalling and undermines sorafenib efficacy[48] aligns with Lou et al's converse finding that engineered vesicles delivering tumor suppressor micro ribonucleic acid 122 can restore sorafenib sensitivity[38]. This dialectical evidence suggests that vesicles constitute both an obstacle and an opportunity in therapeutic modulation. Their endogenous role is to disseminate resistance factors, yet the same pathways can be hijacked to deliver anti-tumor cargo.

This duality is emblematic of biological systems: the very attributes that render vesicles oncogenic in one context provide

therapeutic potential in another. For hepatocellular carcinoma, which remains refractory to many systemic agents, vesicle-based interventions may offer new lines of attack. The challenge lies in distinguishing endogenous vesicle activity, which is predominantly tumor promoting, from engineered therapeutic vesicle activity, which seeks to restore tumor suppressive equilibria.

Elucidating further on the biomarker promise and limitations ,the diagnostic and prognostic utility of vesicle cargo constitutes perhaps the most immediately translatable dimension of this research. Multiple groups, exemplified by Pu et al[25] and Shi et al[31], report vesicle micro ribonucleic acid signatures that outperform alpha fetoprotein and correlate with recurrence risk. Vesicle protein panels[28] and surface marker profiles[29] further extend the biomarker repertoire.

Yet enthusiasm must be tempered by recognition of limitations. Cohort sizes remain modest, often fewer than one hundred patients, raising concerns of overfitting and limited external validity. Many signatures have yet to undergo rigorous prospective validation. Furthermore, confounding factors such as chronic hepatitis, cirrhosis, or metabolic liver disease are rarely disentangled. Since such conditions themselves alter vesicle release, distinguishing malignant from non-malignant vesicle cargo remains a formidable challenge.

The standardization of isolation methods is critical here. The discordance between ultracentrifugation and precipitation methods in terms of vesicle purity[33] means that cargo profiles may vary more as a function of methodology than of disease biology. The field must converge on consensus isolation and normalization strategies to permit genuine biomarker validation.

Perhaps as a con like any literature, this disquisition is not without contradictions. For example, some studies implicate vesicle derived micro ribonucleic acid 21 as oncogenic[25], whereas others suggest it can modulate immune surveillance in ways that may be context dependent[21]. Such apparent paradoxes may reflect differences in vesicle subtype, cellular origin, or microenvironmental context. Tumour heterogeneity further complicates interpretation: vesicles derived from distinct clonal subpopulations may carry divergent cargo with opposing functions.

Another unresolved tension concerns vesicle classification itself. Distinctions between exosomes, microvesicles, and apoptotic bodies remain largely operational rather than mechanistically definitive. Without reliable molecular markers of subtype, attribution of functional roles to specific vesicle categories is tentative at best. This lack of taxonomic clarity hinders mechanistic resolution and complicates therapeutic targeting.

The strength too, of the evidence is variable. Mechanistic studies in cell lines offer molecular precision but limited physiological fidelity. Animal models provide a more complex milieu yet are often immunologically or genetically divergent from human disease. Clinical studies are highly relevant but typically constrained by small scale and retrospective design. As such, each methodological tier contributes partial insights, yet none individually suffice for definitive translation. The most robust conclusions arise when findings converge across tiers, as in the case of vesicle mediated angiogenesis or immune suppression, where mechanistic, preclinical, and clinical data align.

Therefore, Scaling up clinical investigation remains the most pressing requirement. Multi centre prospective studies enrolling diverse populations and employing harmonised protocols are needed to move vesicle biomarkers and therapeutics from experimental promise to clinical reality.

From a translational standpoint, vesicle research intersects with several ongoing trajectories in hepatocellular carcinoma management. Immunotherapy, particularly checkpoint blockade, has shown modest efficacy in subsets of patients. Evidence that vesicle secretion blockade augments checkpoint response[24] suggests a promising combinatorial strategy. Similarly, as precision oncology strives to tailor therapy based on molecular profiles, vesicle cargo signatures could serve as dynamic, non invasive surrogates of tumour biology, guiding therapeutic adjustment in real time.

Therapeutically, vesicle engineering aligns with the broader movement toward biologically inspired nanomedicine. The capacity to load vesicles with micro ribonucleic acids, small interfering ribonucleic acids, or drugs and deliver them with relative biocompatibility is a major advance. Nonetheless, unresolved issues of targeting specificity, large scale production, and regulatory classification must be addressed before clinical application.

6. BROADER CONCEPTUAL IMPLICATIONS

Beyond immediate translational considerations, vesicle biology raises deeper conceptual questions regarding the nature of intercellular communication in cancer. That malignant hepatocytes systematically externalise vesicles carrying regulatory cargo suggests that cancer progression is not merely the accumulation of intracellular mutations but also the construction of extracellular communication networks. Vesicles emerge as vehicles of collective behaviour, enabling tumour populations to coordinate growth, immune evasion, and adaptation. Understanding cancer thus requires not only genomic and proteomic dissection of individual cells but also interrogation of the communicative ecology that sustains malignancy.

A.Placement within the Global Hepatocellular Carcinoma Landscape

Hepatocellular carcinoma represents a malignancy of daunting complexity, arising at the intersection of viral infection,

metabolic injury, and cirrhotic architecture. Traditional biomarkers have underperformed, systemic therapies have limited efficacy, and recurrence remains the rule rather than the exception. In this bleak context, the emergence of extracellular vesicles as both mechanistic drivers and translational tools constitutes a rare frontier of optimism.

Yet optimism must be balanced with caution. Without rigorous methodological consolidation and clinical validation, vesicle research risks remaining an intellectual curiosity rather than a transformative force. The path forward lies in the disciplined integration of vesicle biology into the established frameworks of hepatology and oncology, guided by both scientific ambition and clinical pragmatism.

7. A FINAL INTEGRATED TALK ON LIMITATIONS PRECISE TO THE STUDY

While the integrated body of evidence is compelling, several limitations temper definitive conclusions. Sample sizes are frequently small, with many clinical studies enrolling fewer than fifty patients. This restricts statistical power and generalisability. Moreover, case–control designs predominate, leaving a paucity of prospective longitudinal data that would confirm predictive utility.

Confounding by liver disease background is another recurrent issue. Cirrhosis, viral hepatitis, and non alcoholic steatohepatitis generate vesicle populations that may obscure tumour specific signals. Few studies adequately stratify cohorts by aetiology. The specificity of vesicle biomarkers thus remains uncertain.

Functional studies, though mechanistically illuminating, are often confined to cell lines or xenograft models. These systems may not fully recapitulate the human hepatic microenvironment, which is shaped by chronic inflammation, fibrosis, and metabolic perturbations. Translation from bench to bedside therefore requires cautious interpretation.

Evidence quality mapping indicates that the strongest support exists for vesicle mediated immune modulation and biomarker utility, whereas therapeutic applications remain largely preclinical. Yet the rapid pace of vesicle engineering research suggests that translational milestones may soon be within reach.

8. TRANSLATIONAL PERSPECTIVES AND FUTURE DIRECTIONS

The integration of vesicle research into clinical hepatology demands deliberate progression along several axes as follows:

First, diagnostic translation necessitates standardisation of vesicle isolation and cargo quantification, coupled with validation in large multicentre cohorts. The development of automated, clinically compatible isolation devices would greatly accelerate adoption. Combination panels incorporating vesicle derived micro ribonucleic acids, proteins, and circular ribonucleic acids may surpass the performance of single markers.

Second, therapeutic applications require advances in vesicle engineering. Improvements in loading efficiency, targeting specificity, and scale up manufacturing are essential. Hybrid approaches combining natural vesicle scaffolds with synthetic nanomaterials may provide optimal pharmacokinetics and biodistribution. Regulatory frameworks must evolve to accommodate these bio hybrid therapeutics, which blur traditional categories of biologics and nanomedicines.

Third, the immunological role of vesicles warrants deeper exploration. The intersection of vesicle biology with immune checkpoint pathways is particularly fertile. Clinical trials investigating vesicle blockade as an adjunct to checkpoint inhibition would be a logical progression of current preclinical findings.

Fourth, systems biology approaches should be leveraged to integrate vesicle omics data with genomic, transcriptomic, and metabolomic profiles of hepatocellular carcinoma. This holistic perspective could uncover emergent patterns inaccessible through single modality analysis. Artificial intelligence and machine learning algorithms may prove invaluable in deciphering these complex data landscapes.

And finally, ethical and societal considerations must not be neglected. The use of patient derived vesicles for therapeutic or diagnostic purposes raises questions regarding consent, privacy, and biobanking. Transparent governance frameworks will be essential as vesicle based technologies advance towards clinical implementation.

9. CONCLUSION

Extracellular vesicles occupy a pivotal position in the contemporary understanding of hepatocellular carcinoma. They are not passive byproducts of cellular activity but active agents orchestrating tumour progression, immune modulation, and therapeutic resistance. Simultaneously, they serve as rich reservoirs of diagnostic and prognostic information and as promising vectors for therapeutic delivery.

The twelve studies synthesised in this review exemplify the breadth of vesicle biology, spanning mechanistic dissection, clinical biomarker discovery, and translational innovation. Yet they also highlight the challenges of methodological heterogeneity, limited cohort sizes, and incomplete clinical validation. Addressing these challenges requires concerted multi disciplinary collaboration, harmonisation of protocols, and rigorous clinical testing.

As hepatocellular carcinoma continues to impose a formidable global burden, the investigation of extracellular vesicles offers a new frontier. Their capacity to illuminate the molecular dialogue between tumour and host, and their potential to be harnessed as both biomarkers and therapeutic agents, positions them as central to the next generation of hepatology and oncology. If methodological and translational barriers can be overcome, vesicles may well redefine the diagnostic and therapeutic paradigms of liver cancer in the coming decade..

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