cancers

MDPI

Review

Extracellular Vesicles, Circadian Rhythms, and Cancer: A Comprehensive Review with Emphasis on Hepatocellular Carcinoma

Baharan Fekry [1,*], Lierni Ugartemendia [1], Nestor F. Esnaola [2] and Laura Goetzl [1]

[1] McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030, USA; lierni.ugartemendiaugalde@uth.tmc.edu (L.U.); laura.goetzl@uth.tmc.edu (L.G.)
[2] Division of Surgical Oncology and Gastrointestinal Surgery, Department of Surgery, Houston Methodist Hospital, Houston, TX 77030, USA; nfesnaola@houstonmethodist.org
* Correspondence: baharan.fekry@uth.tmc.edu

Simple Summary: Extracellular vesicles (ECVs), especially exosomes, play a crucial role in hepatocellular carcinoma (HCC) by facilitating intercellular communication and influencing circadian rhythms, thereby affecting HCC progression and treatment responses. Exosomes serve as biomarkers for early cancer detection, drug delivery vehicles, and modulators of immune responses. They promote angiogenesis, modulate the tumor microenvironment, and spread drug resistance. Advanced techniques for isolating cell-specific exosomes and aligning treatments with natural circadian rhythms show promise for early detection and personalized therapies. Understanding how exosomal cargo is sorted and interacts with circadian genes could revolutionize HCC diagnostics and treatments, improving patient outcomes. Utilizing liver-specific proteins for precise exosome isolation can enhance early detection and treatment efficacy, paving the way for more personalized and effective cancer therapies.

Abstract: This review comprehensively explores the complex interplay between extracellular vesicles (ECVs)/exosomes and circadian rhythms, with a focus on the role of this interaction in hepatocellular carcinoma (HCC). Exosomes are nanovesicles derived from cells that facilitate intercellular communication by transporting bioactive molecules such as proteins, lipids, and RNA/DNA species. ECVs are implicated in a range of diseases, where they play crucial roles in signaling between cells and their surrounding environment. In the setting of cancer, ECVs are known to influence cancer initiation and progression. The scope of this review extends to all cancer types, synthesizing existing knowledge on the various roles of ECVs. A unique aspect of this review is the emphasis on the circadian-controlled release and composition of exosomes, highlighting their potential as biomarkers for early cancer detection and monitoring metastasis. We also discuss how circadian rhythms affect multiple cancer-related pathways, proposing that disruptions in the circadian clock can alter tumor development and treatment response. Additionally, this review delves into the influence of circadian clock components on ECV biogenesis and their impact on reshaping the tumor microenvironment, a key component driving HCC progression. Finally, we address the potential clinical applications of ECVs, particularly their use as diagnostic tools and drug delivery vehicles, while considering the challenges associated with clinical implementation.

Keywords: hepatocellular carcinoma; circadian rhythms; cancer biomarkers; extracellular vesicles; exosomes; exosomal cargo; early cancer detection

check for updates

Citation: Fekry, B.; Ugartemendia, L.; Esnaola, N.F.; Goetzl, L. Extracellular Vesicles, Circadian Rhythms, and Cancer: A Comprehensive Review with Emphasis on Hepatocellular Carcinoma. *Cancers* **2024**, *16*, 2552. https://doi.org/10.3390/cancers16142552

Academic Editors: Georgios Germanidis and Dimitris C. Zacharoulis

Received: 20 June 2024
Revised: 12 July 2024
Accepted: 14 July 2024
Published: 16 July 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Extracellular vesicles (ECVs), including exosomes, are secreted by diverse cell types, ranging from fibroblasts to cancer cells, and play a critical role in facilitating the intercellular transmission of signals through bioactive molecules like nucleic acids, proteins, lipids, and metabolites. ECVs can vary in size, content, and surface markers, reflecting the diverse

origins of their parent cells. This heterogeneity influences extracellular vesicle (ECV) function, as different subpopulations may have distinct effects on recipient cells [1–3]. The significance of ECVs is underscored by their ubiquitous presence in diverse body fluids, including bile, blood, breast milk, urine, cerebrospinal fluid, and saliva [4–6].

The circadian rhythm functions as an internal biological clock, regulating physiological activities throughout a 24 h period. The circadian rhythm's impact on cellular activities extends to the synthesis and packaging of ECV cargo, influencing variations in its composition throughout the day [7–11]. Disruptions to the circadian clock have been implicated in tumor development, progression, and responses to treatment across different types of cancers [12–16], which has a bearing on tailoring targeted therapeutic strategies to specific types of cancer [17,18]. Early detection and treatment of cancer is pivotal for enhancing patient outcomes. Therefore, sensitive and specific biomarkers are critical in early cancer detection and the monitoring of progression and treatment response. The sensitivity and specificity of the biomarkers currently available for cancer detection are inadequate, especially in accurately distinguishing between individuals at high or low risk for the disease [19–23]. Given their distinct composition and rich biological data, ECVs are an innovative and novel source of cancer biomarkers. One emerging barrier is their heterogeneity, with diverse subpopulations of tumor-derived ECVs playing varying roles in progression and metastasis [24–28].

Hepatocellular carcinoma (HCC) comprises over 90% of liver cancers and ranks as the fifth most common cancer and the third leading cause of cancer death worldwide, with 840,000 new cases and 780,000 deaths annually [29,30]. Underlying tissue alterations from hepatitis contribute to late-stage detection (85% at intermediate-to-advanced stages). Patients with early-stage disease are eligible for liver-directed therapies (including ablation), surgical resection, and liver transplantation [31]. Five-year survival with these potentially curative therapies can exceed 70%; in contrast, five-year survival after palliative therapies for advanced-stage disease is generally less than 5% [32,33]. Due to suboptimal surveillance methods for HCC (i.e., abdominal imaging with ultrasound, computed tomography, or magnetic resonance imaging) and persistent poor utilization and uptake among high-risk populations, most patients, unfortunately, present with advanced disease [32]. The only biomarker currently validated for clinical use for screening for HCC, alpha-fetoprotein (AFP), has marginal sensitivity and specificity (approximately 60 and 80%, respectively); not surprisingly, most societal guidelines recommend its use only in combination with abdominal ultrasound [32,34–36]. Other biomarkers that have been tested alone and in combination with AFP in large retrospective longitudinal studies, such as AFP-L3 and DCP, have similarly marginal performance characteristics [37]. As such, an urgent need exists for newer, more accurate, non-invasive biomarkers for early detection and diagnosis of HCC that do not necessarily need to be used in combination with abdominal imaging (which adds cost and impairs more widespread uptake, particularly across under-resourced settings). Several studies have indicated a close association between ECVs and the onset and progression of HCC. A thorough investigation into ECVs, including circadian influences, may reveal insights into tumor formation and metastasis mechanisms, offering novel approaches to early diagnosis and treatment [38–40].

2. Exosome Biogenesis

Traditional extracellular vesicles (ECVs) include exosomes (40–150 nm), microvesicles, and apoptotic bodies. Recent research has identified additional types, such as autophagic ECVs, stress-induced ECVs, and matrix vesicles [41–43]. The formation of exosomes occurs through a complex and highly regulated process, where intraluminal vesicles (IVLs) are created through inward budding endosomal membranes, with the sorting and enrichment of exosome components; exosomes are then disgorged from multivesicular bodies (MVBs) [44,45] (Figure 1).

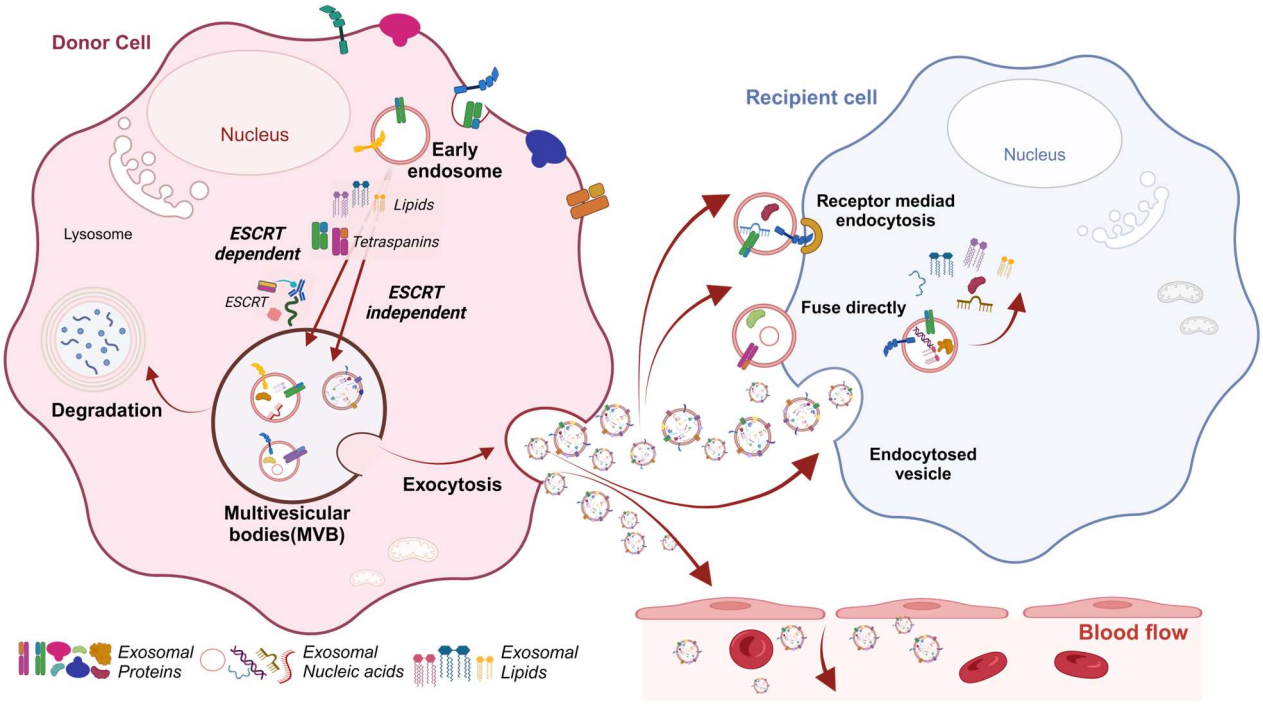

Figure 1. Exosome biogenesis. The primary pathway of exosome biogenesis involves the creation of multivesicular bodies (MVBs) within endosomes, leading to exosome secretion. During endocytosis, early endosomes transform into MVBs, generating intraluminal vesicles (ILVs) through the inward budding of their membranes. These MVBs may either merge with lysosomes for degradation or move to the cell surface to release exosomes. This release process is facilitated by RAB GTPases and SNARE complexes. Once exosomes are released, extracellular vesicles (ECVs) can interact with target cells through ligand–receptor binding, endocytosis, or membrane fusion, allowing the delivery of their cargo into the cytoplasm of the recipient cell. The molecular content of ECVs plays a crucial role in regulating various functions in target cells, including intracellular signaling, gene regulation, and metabolism, and can also be directly released into biological fluids. Created with BioRender.com.

Exosomes may release their material into the intracellular environment or internalize it into early endosomes. Early endosomes mature and form exosome-like vesicles, which can either be recycled and resecreted or degraded after fusion with a lysosome, supporting the recipient cell's metabolism [45,46]. The genomic health of cells and distinct cell types can further influence the regulation of exosome biogenesis [47]. Exosomes are formed through two pathways: the endosomal sorting complex required for transport (ESCRT)-dependent pathway, and the ESCRT-independent pathway. The ESCRT-dependent pathway is considered the most important mechanism to sort cargoes and mediate membrane shaping for exosome formation. Numerous molecules, including the ESCRT complex, are involved in the sorting of ubiquitinated proteins into ILVs through this pathway. However, in the ESCRT-independent pathway, MBV and ILV formation occurs through the sphingomyelinase enzyme, using chaperones and tetraspanins (CD63 or CD81) to assist in cargo binding and membrane formation. Exosomal proteins, either luminal or surface-bound, enable subtyping with surface markers like CD9, CD63, and CD81, which are integral to exosome formation and function. Proteins such as GTPases, Annexins, and Rab GTPases facilitate endosomal processes and vesicle trafficking. Glycoproteins like β-galactosidase improve the exosome-targeting accuracy [48–57]. Exosomes also exhibit abundant nucleic acids, indicating an active sorting mechanism during their biogenesis [58], such as DNA, mRNA, and non-coding RNA (ncRNA) species [57,59]. MicroRNAs (miRNAs) stand out as one of the most abundant RNA species in exosomes, playing diverse roles in biological processes that are especially relevant to cancer, such as exocytosis, hematopoiesis, and angiogenesis, contributing to exosome-mediated cellular communication. Additional exosomal RNA

species, like long ncRNA (lncRNA), circular RNAs (circRNAs), or small nuclear RNA, also impact biological processes, particularly influencing tumor development [60,61]. Exosomes affect target cells by binding to plasma membrane receptors or internalizing and releasing their contents, altering cellular processes and metabolism [45,46]. Current evidence suggests the selective packaging of ECV cargo, as shown by significant protein and RNA level variability compared to parental cells [62,63]. While the exact cargo-sorting mechanisms are unclear, RNA-binding proteins, Rab GTPases, and post-translational modifications, like ubiquitination and phosphorylation, are implicated [64]. Factors such as endoplasmic reticulum stress or phenotypic activation can influence ECV abundance and composition [65]. Current research focuses on the potential applications of these exosomal components as non-invasive biomarkers for disease diagnosis and prognosis.

3. The Role of Exosomes/ECVs in Oncogenesis

ECVs/exosomes play a critical role in the oncogenic process, facilitating the transfer of nucleic acids and proteins that disrupt cellular homeostasis and stimulate cancer initiation, progression, and metastasis. ECVs can carry oncogenic signatures that have the potential to transform recipient cells by altering gene expression and inducing malignant transformations [24,66,67].

Exosomes modulate immune responses, facilitating tumor cell evasion of immune surveillance and promoting metastatic spread. ECVs can transport immunosuppressive molecules that inhibit cytotoxic T cells and natural killer cells, allowing tumor cells to proliferate unchecked. Exosomes can also enhance metastasis by restructuring the extracellular matrix, which enables tumor cells to invade adjacent tissues and migrate to distant organs. Additionally, they play a vital role in the horizontal transfer of drug-resistance traits among cancer cell populations, spreading a chemoresistance that makes treatment more challenging. Clinically, exosomes can be extracted from body fluids like blood or urine and have the potential to monitor cancer non-invasively. The molecular composition of exosomes reflects the pathological state of their cells of origin, providing insights that are crucial in early detection, tracking disease progression, or adjusting therapeutic strategies. Through the analysis of exosomal biomarkers, clinicians can identify specific molecular signatures indicative of cancer presence, evaluate the aggressiveness of the disease, identify drug resistance, and monitor therapeutic response. This capability is essential in implementing personalized treatment plans that optimize therapy effectiveness and improve overall patient prognosis, making exosomes a cornerstone of modern oncological research and treatment strategies [24,48,66–69]. Table 1 aggregates the changes in exosomal ncRNA levels across various cancer types, revealing how these molecules could serve as biomarkers for diagnosis, prognosis, and treatment monitoring, particularly highlighting the extensive range of applications in diverse cancer contexts.

Table 1. Exosomal ncRNAs as potential cancer biomarkers in diagnosis and progression.

Exosomal ncRNA	Type of Disease	Source of Exosome	Expression	References
miRNA-21, miRNA-1224, miRNA-1229, miRNA-1246, miRNA-150, miRNA-21, miRNA-223, miRNA-23a	Colorectal cancer	Blood	↑, ↑, ↑, ↑, ↑, ↑, ↑, ↑	[70,71]
miRNA-21, miRNA-105, miRNA-372	Breast cancer	Blood	↑, ↑, ↑	[72,73]
miRNA-373	Triple-negative breast cancer	Blood	↑	[74]
miRNA-21, miRNA-141, miRNA-200a, miRNA-200c, miRNA-200b, miRNA-203, miRNA-205, miRNA-214	Ovarian cancer	Blood	↑, ↑, ↑, ↑, ↑, ↑, ↑, ↑	

Table 1. *Cont.*

Exosomal ncRNA	Type of Disease	Source of Exosome	Expression	References
miRNA-17-5p, miRNA-21, miRNA-106a, miRNA-106b	Pancreatic cancer	Blood	↑, ↑, ↑, ↑	[75,76]
miRNA-184	Tongue cancer	Blood	↑	[77]
miRNA-21, miRNA-500, mi-RNA-26a, mi-RNA-26c, miRNA-224, miRNA-665, miRNA-10b-5p, miRNA-18a-5p, miRNA-215-5p, miRNA-940mi-RNA-199a, miRNA-18a, miRNA-221, miRNA-222	Hepatocellular carcinoma	Blood	↑, ↓, ↓, ↓, ↑, ↑, ↑, ↑, ↑, ↑, ↑, ↑, ↑	[78–82]
miRNA-125a, miRNA-200a	Oral squamous cell carcinoma	Blood	↓	[83]
circ-DB, circCUHRF1, circTMEM4A, circ-100338, circ-0051443	Hepatocelluar carcinoma	Blood	↑, ↑, ↑, ↑, ↓, ↓	[84–87]
circ_0047921, circ_0056285, circ_0007761	Non-small cell lung cancer	Blood	↓, ↓, ↑	[88]
circ_0001439, circ_0001492, circ_0000896	Lung adenocarcinoma	Blood	↑, ↑, ↑	[89]
circMYC	Nasopharyngeal carcinoma	Blood	↑	[90]
circ_0047921, circ_0056285, circ_0007761	Non-small cell lung cancer	Blood	↓, ↓, ↑	[88]
hsa_circ_0055202, hsa_circ_0074920 hsa_circ_0043722	Glioblastoma	Blood	↑, ↑, ↑	[91]
circPDLIM5	Prostate cancer	Urine	↑	[92]

↑ up ↓ down.

3.1. Hepatic-Cell-Derived ECVs: Cargo and Functions

Approximately 80% of the liver's volume is made up of hepatocytes, which are crucial in physiological processes, while 6.5% consists of non-parenchymal cells, like liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), cholangiocytes, and Kupffer cells. Minority cell populations support hepatocytes and maintain the hepatic environment. The liver's lobular structure promotes effective bidirectional intercellular communication and molecular information transfer through ECVs (Figure 2). For example, hepatocyte-derived ECVs carry arginase-1, which modulates endothelial cell function, and sphingosine-1-phosphate, which promotes liver regeneration. LSEC-derived ECVs modulate hepatic stellate cell activity, and HSC-derived ECVs may facilitate HCC progression. Cholangiocyte-derived ECVs affect bile acid homeostasis and promote healing. This encapsulates the varied currently known roles and cargo of hepatic-cell-derived ECVs in liver function and disease [93–101].

In addition to playing a direct role in HCC, ECVs from hepatocytes and adipocytes carry specific cargoes that enhance steatosis and immune activation, driving the progression of chronic liver diseases like non-alcoholic fatty liver disease and alcoholic liver disease and ultimately increasing the downstream risk for HCC [102]. For example, hepatocyte-derived ECVs contain miRNAs such as miRNA-122, miRNA-192, and miRNA-128-3p, as well as mitochondrial DNA, influencing inflammation and metabolic dysregulation in non-alcoholic liver disease [103,104]. In alcoholic liver disease, hepatocyte-derived ECVs carry CD40 ligand and mitochondrial DNA, promoting inflammation and immune cell activation [105]. However, adipocyte-derived ECVs carry miRNA-99b target hepatocytes to

promote steatosis [106]. Additionally, circulating ECVs in HCC display altered expression of surface markers, including Annexin V, EpCAM, ASGR1, and CD133, offering a panel of markers to distinguish HCC from cholangiocarcinoma and other malignancies [107,108].

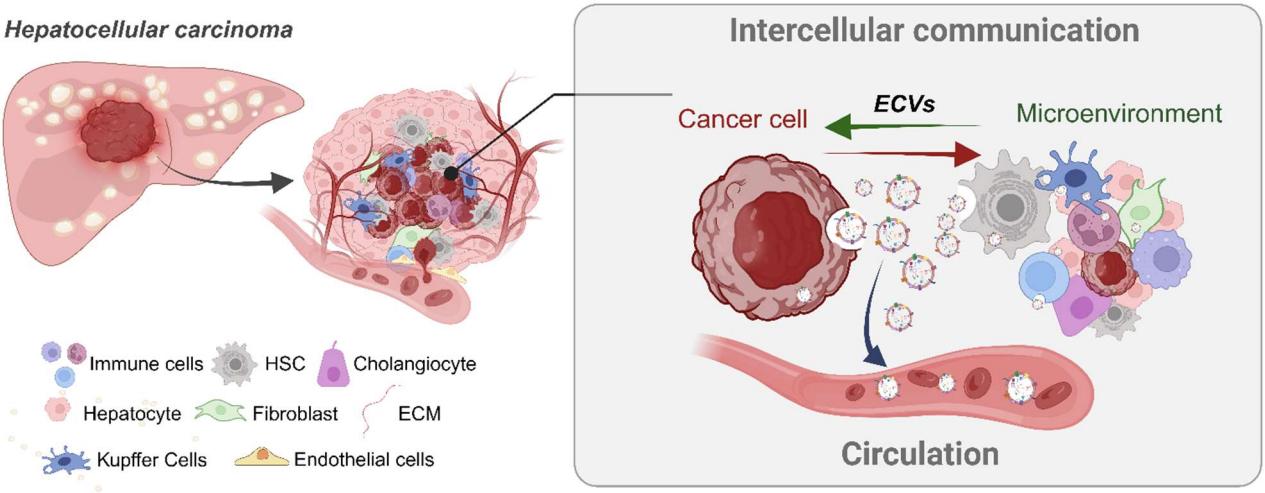

Figure 2. Impact of extracellular vesicles (ECVs) in the hepatocellular carcinoma (HCC) tumor microenvironment. ECVs are released by both tumor cells and other surrounding cells into the tumor microenvironment and the circulatory system. These ECVs transport a variety of bioactive molecules that contribute to tumor growth, metastasis, and intercellular communication. By mediating these exchanges, ECVs play a key role in shaping the tumor microenvironment, potentially influencing the progression of cancer and the behavior of distant cells through the molecules that they carry. ECVs, extracellular vesicles; HSCs, hepatic stellate cells; ECM, extracellular matrix. Created with BioRender.com.

3.2. Exosomes in HCC Initiation, Progression, Metastasis, and Angiogenesis

In HCC, ECVs, particularly exosomes, play a fundamental role in initiation, progression, and metastasis. These vesicles facilitate complex signal transductions within the tumor microenvironment, significantly enhancing cellular communication between tumor cells and surrounding non-tumor cells. ECVs transport a variety of bioactive molecules, including miRNAs and proteins, which are instrumental in promoting cellular transformations, inflammatory responses, and adaptive mechanisms essential to tumor survival and expansion. Through their cargo, ECVs upregulate inflammatory cytokines and support adaptive responses under hypoxic conditions, contributing to tumor growth and the spread of cancer cells. They also induce critical processes such as epithelial–mesenchymal transition (EMT) and manipulate the immune landscape, enhancing the tumor's invasiveness and capacity to evade immune detection [38,109]. Additionally, the acidic microenvironment in HCC further stimulates ECVs to strengthen the expression of miRNAs that promote cell proliferation and invasion, intensifying the aggressiveness of cancer [110]. Table 2 contains selected examples and emphasizes the various roles of ECVs in the context of HCC.

Table 2. Role of ECVs in HCC carcinogenesis.

Exosomal Component	Effect	Mechanism of Impact	References
miRNA-93	Tumor proliferation	Enhances the growth of HCC cells	[111,112]
PDGFRα, Hedgehog ligands	Fibrogenesis, angiogenesis	Promotes tissue remodeling and blood vessel formation	[64]
miRNA-1247-3p	Lung metastasis	Converts cells to cancer-associated fibroblasts and increases pro-inflammatory cytokines (IL-6, IL-8)	[18]

Table 2. *Cont.*

Exosomal Component	Effect	Mechanism of Impact	References
Circ-0004277, miRNA-136-5p	EMT, tissue invasion	Induces epithelial-to-mesenchymal transition in surrounding cells	[113,114]
miRNA-21, miRNA-10b	Tumor proliferation, metastasis	Regulation of the tumor suppressor gene PTEN through modulation of Tet methylcytosine dioxygenase expression. Increases HIF-1α mRNA and promotes survival under hypoxia	[110]
miRNA-155	Inflammation	Intensifies IL-6 and IL-8 levels and enhances inflammatory response	[115,116]
GOLM1	Tumor occurrence, metastasis	Activates GSK-3β/MMP signaling, a potential biomarker	[38,117]
miRNA-320a, miRNA-451a	Anti-tumorigenic effects	Inhibits proliferation, migration, angiogenesis	[118,119]
LncRNA H19	Enhanced angiogenesis	Increases VEGF secretion and VEGF-R1 production	[120]
miRNA-32	Angiogenesis	Suppresses PTEN and activates PI3K/Akt pathway	[110]
VASN	Tumor development, angiogenesis	Stimulates endothelial cell proliferation and neovascularization	[121]
miRNA-200b-3p	Anti-angiogenic	Inhibits erythroblast transformation-related genes	[122]
HMGB1	Immune evasion	Expands regulatory B cells, facilitating tumor survival	[123]
Exosomal SMAD, Caveolin, MET, caveolins, S100, ITGαvβ5, OXL4, SDF-1α, IL-6, IL-8, AFP, and GGT	HCC progression	Cell adhesion, motility, invasive abilities, metastasis, angiogenesis, and cell proliferation	[124–128]
miRNA-92a-2-5p	Promotes metastasis	Enhances cancer cell invasion via AR/PHLPP/p-AKT/β-catenin	[129]
miRNA-125a/b	Impedes tumor-associated macrophages	Targets CD90 in tumor-associated macrophages	[130]

3.3. ECVs and Tumor Microenvironment

ECVs, including exosomes, are critical mediators within the tumor microenvironment, which is essential for tumor progression and metastasis. In HCC, the tumor microenvironment often becomes acidic and hypoxic, influencing the behavior of ECVs. This acidic environment arises due to inadequate perfusion in early tumors, leading to hypoxia and a reliance on anaerobic metabolism, which elevates lactate production and acidifies the tumor microenvironment [131]. This metabolic reprogramming, known as the "Warburg effect", occurs even in the presence of sufficient oxygen. Due to these acidic conditions, ECV levels increase and enhance the proliferation, migration, and invasion of recipient cells [132]. Moreover, hypoxia modulates the expression of cell surface receptors and ceramides through hypoxia-inducible factors, further facilitating tumor metastasis [133,134].

ECVs and various non-immune cells in the tumor microenvironment, such as cancer-associated fibroblasts (CAFs), endothelial cells, and adipocytes, play a crucial role in tumor progression and metastasis. CAFs, arising from diverse cell origins, including fibroblasts and mesenchymal stem cells, are pivotal in the microenvironment, as they deposit extracellular matrix proteins and facilitate tumor invasion. Studies have shown that ECVs released by highly metastatic HCC cells contain miRNA-1247-3p, which can transform normal fibroblasts into CAFs, enhancing metastasis through the secretion of pro-inflammatory cytokines. Conversely, CAF-derived ECVs with reduced levels of miRNA-150-3p have been found to suppress the migration and invasion of HCC cells [135,136]. Cargo from

endothelial-cell-derived ECVs facilitate angiogenesis, enhancing HCC proliferation and metastasis [86,120,122,137,138]. Additionally, adipocytes within the tumor microenvironment, particularly cancer-associated adipocytes, contribute to tumor progression by creating a hypoxic environment, promoting inflammation, and remodeling the extracellular matrix. These interactions between ECVs and the tumor microenvironment highlight the complexity of HCC pathogenesis, emphasizing the need for targeted therapeutic strategies. Moreover, ECVs' role within the tumor microenvironment is crucial in advancing both cancer treatment and diagnostics.

3.4. Diagnostic Biomarkers in HCC

Due to the absence of reliable biomarkers, the early diagnosis of HCC is challenging, with most cases identified at advanced stages [34]. There is growing interest in potentially using "liquid biopsies" for the early detection of HCC [139,140]. Although several hypermethylated target genes identified in circulating cell-free DNA have been shown to be specifically associated with HCC, their expression in the peripheral blood is very low, particularly in the setting of early-stage disease [139]. Non-coding microRNAs and long non-coding RNAs, which play regulatory roles in various diseases and cancers, have also been investigated as potential biomarkers for HCC. Although their utility is somewhat limited due to differences across ethnic populations and underlying etiologies of HCC, studies have suggested that when used in combination with each other, as well as with AFP, they can show an excellent diagnostic performance (i.e., area under the curve (AUC) > 90%) particularly when enriched within exosomes (rather than when detected in exosome-depleted serum fractions) [141–144].

ECVs are promising diagnostic markers for cancer due to their lipid bilayer, which preserves crucial biomolecules and ensures stability in bodily fluids, enabling minimally invasive and more reliable detection [145]. This resilience ensures that both fresh and long-term-stored exosome samples maintain important tumor information for analysis [67,146–148].

Exosomes in HCC have potential clinical applications, as they promote immune escape, angiogenesis, metastasis, and tumor invasion. They can also confer drug resistance and modulate immunotherapy responses. Exosomes may serve as biomarkers for detecting and monitoring HCC, highlighting their relevance to targeted therapies and personalized medicine (Figure 3). In addition to total circulating ECV markers, specific cell membrane proteins can integrate into secreted ECV membranes [149]. These cell-type-specific surface proteins aid in the immunoaffinity-based isolation of ECVs from particular cells or tissues, enhancing disease biomarker detection sensitivity and specificity. Goetzl et al. have pioneered a novel method in the field of non-invasive biomarkers by isolating fetal central-nervous-system-derived exosomes from maternal blood. This innovative technique enables the detection and characterization of fetal brain injuries through a non-invasive approach, significantly advancing prenatal diagnostics and monitoring [150–153]. Similarly, research has demonstrated the efficacy of using asialoglycoprotein receptor 1 (ASGR1) to purify hepatocyte-derived exosomes, significantly improving liver disease biomarker detection [154]. In the context of HCC, isolating HCC-derived ECVs is critical in developing precise biomarkers, offering a targeted approach compared to total circulating ECVs. Mass spectrometry of exosomal proteins from HCC cell lines has identified approximately 1400 proteins that facilitate intercellular communication and correlate with clinical parameters, such as tumor size, TNM stage, portal vein tumor thrombosis, and overall survival. This targeted isolation provides superior diagnostic and prognostic capabilities compared to conventional biomarkers like alpha-fetoprotein (AFP), enhancing the specificity and sensitivity of disease detection and enabling more effective personalized treatment strategies for HCC patients [155,156]. Also, it has been shown that the quantity of serum ECVs notably increases during the cirrhotic stage and early stages of HCC compared to healthy liver tissue [157,158], suggesting a potential role of HCC-derived ECVs in the early detection of HCC [148].

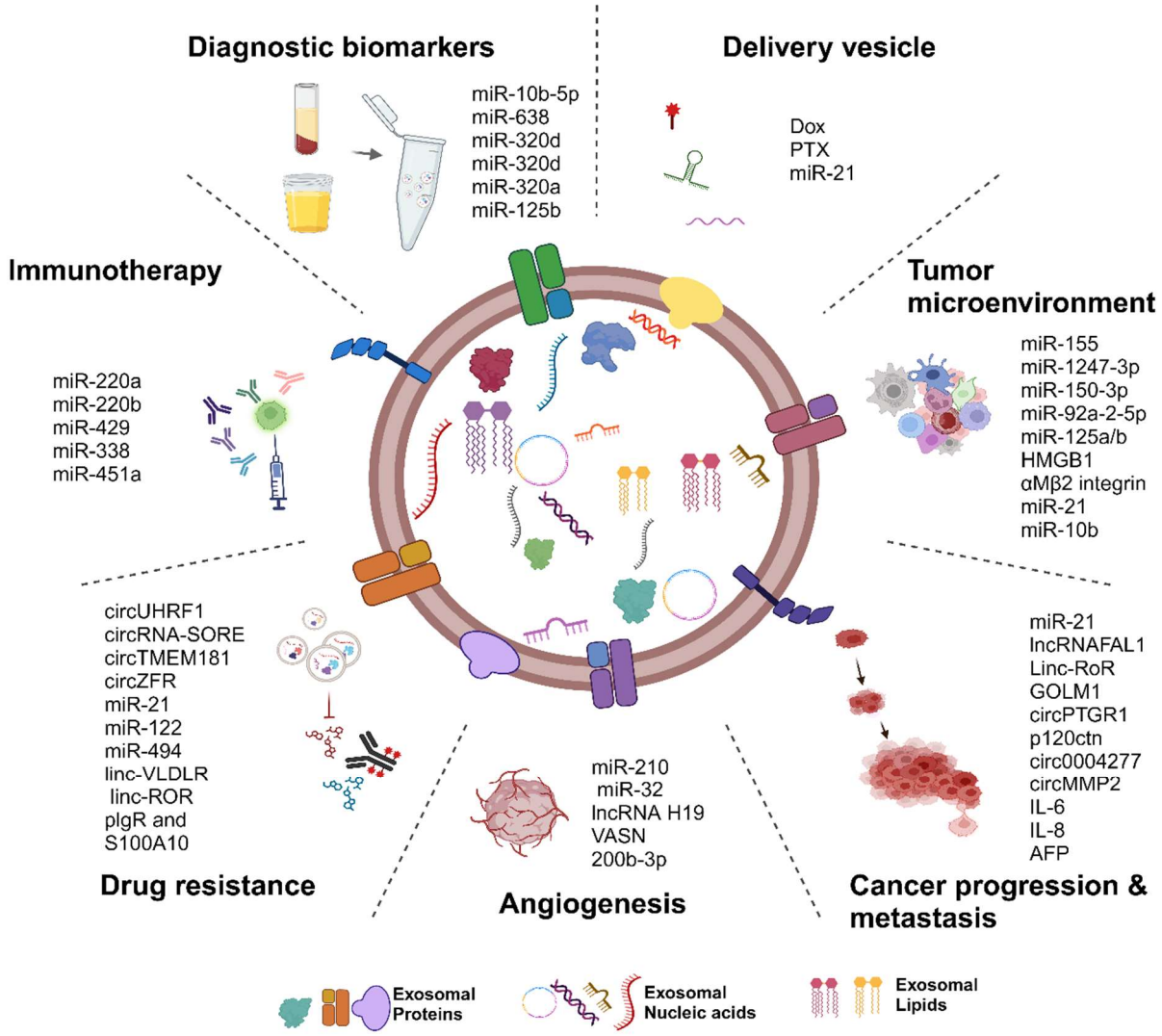

Figure 3. Roles of exosomes in HCC. This diagram demonstrates the multiple functions of exosomes in HCC, highlighting their roles in facilitating immune escape, promoting angiogenesis, driving metastasis, and supporting tumor invasion. Exosomes from other sources, like stem cells and macrophages, can also influence HCC by conferring drug resistance and modulating responses to immunotherapy. These exosomes have additional potential as biomarkers for detecting and monitoring HCC, shedding light on disease progression and offering new pathways for treatment. The ability of exosomes to mediate intercellular communication within the tumor microenvironment emphasizes their relevance to targeted therapies and personalized medical approaches. Created with BioRender.com.

Lipids play a dual role in exosomes. They act as structural components of the exosomal membrane and protect the cargo from peripheral degradation. They are essential contributors to exosome formation and release. It is common to see enrichment of specific lipids in ECVs, creating a "lipid signature" that could be used for diagnosis/prognosis in HCC [159,160]. For example, HepG2/C3a-cell-derived ECVs have specific lipid profiles with higher free cholesterol, ceramides, phosphatidylserine, and sphingomyelin but lower phosphoinositide levels [161–163]. A clinical study showed significant differences in lipid profiles between HCC and non-HCC patients, identifying higher levels of sphingosines, dilysocardiolipins, and (O-acyl)-1-hydroxy fatty acids with HCC, highlighting their potential as non-invasive diagnostic biomarkers [164].

Several studies have highlighted the potential of exosomal miRNAs as early diagnostic biomarkers from HCC cells. Exosomal miRNAs offer superior stability and reduced susceptibility to interference from other blood components, enhancing their biomarker

potential compared to serum-free miRNAs [165–167]. Hyo et al. identified exosomal miRNA-10b-5p as a promising biomarker (AUC, 0.934; sensitivity, 90.7%; specificity, 75.0%; cutoff, 1.8-fold). Another study advocated for the combination of exosomal miRNA-466-5p and miRNA-4746-5p, achieving an AUC of 0.947 (CI, 0.889–0.980; sensitivity, 81.8%; specificity, 91.7%) [167–169]. Decreased miRNA-638 levels in HCC patient serum correlate with adverse tumor characteristics, while elevated levels correlate with improved survival [170]. Additionally, exosomal miRNA-320d levels in serum samples could be used to distinguish HCC patients from healthy controls. Decreased exosomal miRNA-320d levels were associated with advanced tumor stage and positive lymph node metastasis and, therefore, with shorter overall and disease-free survival, indicating poor prognosis in HCC [171].

While the studies on exosomal DNA in HCC are limited, researchers are currently focusing on its potential in tumor diagnosis [38,172]. Yan et al. discovered a significant elevation in the cell-free DNA levels of HCC patients compared to non-HCC patients. The isolation and analysis of disease-specific ECVs represent significant advancements in biomarker research, improving the accuracy and efficacy of non-invasive diagnostics and personalized medicine.

3.5. ECVs in Immunotherapy and Therapy Resistance

Exosomes and ECVs significantly influence HCC therapy through their dual roles in immune modulation and drug resistance. These vesicles regulate immune responses that are crucial to personalized cancer treatment, notably via interactions with the PD-1/PD-L1 axis [173,174]. Additionally, ECVs can act as precise drug delivery vehicles, enhancing the targeting and dosage customization of treatments like Doxorubicin and Paclitaxel, thus reducing toxicity while improving effectiveness [175–179].

Beyond PD-1/PD-L1 inhibition therapy, ECVs also impact the efficacy of other immune checkpoint inhibitors. Tumor-derived ECVs can carry ligands for these checkpoints, modulating the immune environment and contributing to immune evasion by tumors [180–182].

Although primarily used for hematologic malignancies, recently, CAR T-cell therapy has been explored for solid tumors, like HCC. ECVs can modulate the tumor microenvironment, enhancing CAR T-cell persistence and functionality [183]. For instance, mesenchymal-stem-cell-derived ECVs have been shown to enhance CAR T-cell anti-tumor activity by modulating the immune environment. Additionally, ECVs from HCC cells can carry immunosuppressive molecules, such as TGF-β and IL-10, inhibiting CAR T-cell activity [182,184,185]. Engineering ECVs to carry pro-inflammatory cytokines or immune checkpoint inhibitors can potentially counteract this suppression and enhance the efficacy of CAR T-cell therapy.

Dendritic-cell-derived ECVs are particularly promising as immunotherapeutic agents. These ECVs contain immunostimulatory components, functioning as antigen-presenting entities. They can promote an immune-cell-dependent tumor rejection response by enhancing the activation of CD8+ T cells and remodeling the tumor microenvironment. Tumor-derived ECVs also stimulate anti-tumor immune responses and deliver tumor-associated antigens to dendritic cells, enabling efficient antigen presentation on major histocompatibility complex (MHC) molecules to T cells. Inspired by CAR T-cell therapy, researchers have used dendritic-cell-derived ECVs to present MHC–antigen complexes, triggering effective anti-tumor immunity. These engineered ECVs can offer an effective alternative to CAR T cells by promoting T-cell binding to cancer cells [183,186].

Moreover, engineered ECVs are emerging as a promising therapeutic strategy for tumor immunomodulation. For example, exoASO-STAT6 uses ECVs to deliver antisense oligonucleotides that disrupt STAT6 signaling in tumor-associated macrophages. This method has shown strong anti-tumor activity in preclinical models and is currently being tested in clinical trials for advanced HCC [187–189].

Another critical function of ECVs is mediating resistance to therapies, particularly chemotherapy. They transport molecules, such as specific proteins or RNA, that induce drug resistance, spreading it throughout the tumor. Identifying biomarkers within exosomes that signal resistance allows clinicians to develop tailored treatment plans. These plans

might include agents that sensitize tumors to intended therapies, boosting the efficacy of standard treatments without increasing side effects [84,190–195].

Exosomes' capability to carry both water-soluble and lipid-soluble drugs highlights their potential to expand the effectiveness of various cancer therapies. Their versatility and compatibility with biological systems make them valuable in precision medicine, aiming to customize cancer treatments to individual patient profiles for better outcomes [196,197].

Moreover, ECVs have the capacity to enhance and modulate immune responses, positioning them as a promising strategy for novel vaccine formulations. ECVs can potentially activate granulocytes or natural killer cells and interact with CD8, CD4, and B cells, eliciting antigen-specific immune responses. The combination of ECVs with antigens may induce a humoral immune response comparable to that elicited by the antigen alone. This finding suggests that ECVs could serve as vaccine adjuvants, enhancing the efficacy of vaccines [187,188,198].

Recent clinical trials by Escudier et al. and Morse et al. have focused on dendritic-cell-derived exosomes, demonstrating their potential in advancing cancer therapies. These studies show promising results in terms of both safety and efficacy. The positive outcomes indicate the feasibility of integrating exosome-based approaches into standard cancer treatment protocols, leading to more personalized and effective strategies [196,197,199]. Table 3 provides additional details on the role of ECVs in enhancing immunotherapy and managing therapy resistance, showcasing their multifaceted contributions to improving HCC treatment. Exosomes and ECVs are crucial in advancing HCC therapy. Their functions in immune modulation, drug delivery, and overcoming resistance position them at the forefront of personalized cancer treatment strategies, with ongoing research and clinical trials continually revealing their full potential.

Table 3. ECVs in enhancing immunotherapy and managing therapy resistance.

Function	Example	Description	Context and Patient Use	Reference
Immunomodulation	PD-L1-enriched exosomes	Exosomes carrying PD-L1 inhibited T-cell activity, modulating the immune environment to favor tumor growth and impacting responses to immunotherapy	Oncology immunotherapy research—research phase	[174]
Drug Resistance	Exosomes carrying miRNA-1247-3p	Enhanced resistance to sorafenib in HCC by altering gene expression related to drug metabolism and cellular survival pathways	Chemoresistance mechanisms study—research phase	[135]
Chemotherapy Delivery	Exosomes for Doxorubicin delivery	Facilitated targeted delivery of Doxorubicin, enhancing drug specificity and minimizing off-target effects	Targeted chemotherapy research—early clinical trials	[178,179]
Gene Therapy	miRNA-220a/220b/429 mimics	Delivered miRNAs that regulate oncogenic pathways, providing a method for precision gene therapy	Gene therapy innovation—research phase	[174]
Chemoresistance Mechanisms	Exosomes carrying circRNA-SORE and miRNAs in chemoresistance	Studied circRNAs and miRNAs that enhance cellular mechanisms of resistance to chemotherapy agents	Molecular oncology exploration—research phase	[84,190–195]
Enhanced Immune Surveillance	Exosomes carrying HMGB1	Examined the role of HMGB1-bearing exosomes in modulating immune surveillance in HCC	Immune surveillance enhancement—research phase	[176]
Prognostic Biomarkers	Circulating exosomal PD-L1 levels	Developed and validated exosomal PD-L1 as a biomarker for assessing responses to immunotherapy	Biomarker development—early clinical trials	[174]
Therapeutic Drug Delivery	Exosomes in targeted drug delivery	Employed engineered exosomes for specific drug conveyance to tumor sites, reducing systemic toxicity	Drug delivery system development—early clinical trials	[175–179]
Circadian Influence on Therapy	Variability in exosome release by circadian rhythms	Circadian rhythms affect the secretion and composition of exosomes, influencing the response	Chronotherapy research—research phase	[8,200]

4. The Molecular Basis of Circadian Rhythms in Mammals

Mammals synchronize their circadian rhythms primarily with the light–dark cycles in their environment, a process mediated by ocular photoreception that relays signals to the suprachiasmatic nucleus of the hypothalamus. This region coordinates the synchronization of circadian clocks across the body's various tissues. Peripheral clocks are entrained by signals such as humoral cues, metabolic factors, and body temperature fluctuations [201–203]. At the molecular level, the circadian clock is driven by autoregulatory transcription–translation feedback loops (TTFLs) involving transcription factors like brain and muscle ARNT-like 1 (*BMAL1*) and circadian locomotor output cycles kaput (*CLOCK*). These proteins form the CLOCK-BMAL1 heterodimer that binds to E-Box sequences, promoting the expression of clock-regulated genes, including Period (PER) and Cryptochrome Circadian Regulator (CRY). PER and CRY proteins inhibit *BMAL1* activity, and their stability is regulated by ubiquitin ligases and kinases, establishing a new oscillatory cycle. Additional regulators such as REV-ERBs and retinoic acid receptor-related orphan receptors create another feedback loop by repressing or activating *BMAL1* expression, respectively [204,205] (Figure 4). Other molecular oscillators can also function independently of the transcription-based clock in various species [201,206].

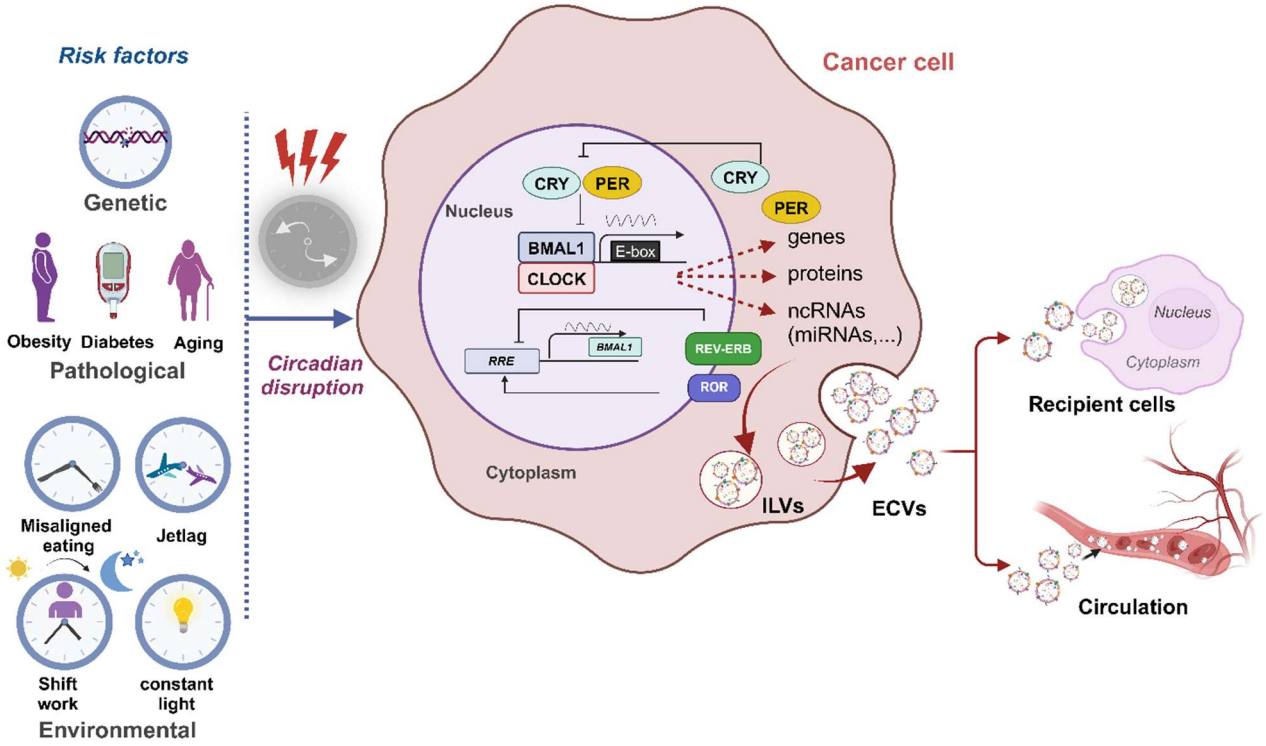

Figure 4. Circadian clock's impact on ECV characteristics in cancer. In the molecular clock, CLOCK and BMAL1 rhythmically bind to E-boxes, activating clock-controlled genes and other clock components. Their activity is regulated by PER and CRY proteins, which inhibit CLOCK-BMAL1 upon entering the nucleus. A second feedback loop involves *BMAL1* transcription, which is repressed by REV-ERB and activated by ROR. The circadian clock plays a crucial role in shaping the properties of ECVs, which are essential for cellular communication and metabolic regulation. Disruptions in circadian rhythms, resulting from factors such as genetic mutations in clock genes; environmental stresses like irregular eating times, jet lag, shift work, and constant light exposure; and pathological conditions like aging, diabetes, and obesity can drive cancer development and progression. These disturbances in circadian patterns significantly affect ECV characteristics, including size, concentration, and cargo composition, emphasizing the critical relationship among circadian rhythms, ECV biology, and cancer dynamics, whether through circulation or interactions with recipient cells. Created with BioRender.com.

Circadian disruptions stem from environmental, genetic, and pathobiological factors. They influence the body's internal clocks, disturbing critical physiological functions, such as sleep, alertness, motor skills, body temperature regulation, urinary system functionality, hormone secretion, immune responses, cytokine release, and cell cycle progression [207–209]. Shift work, jet lag, exposure to artificial light at night, irregular meal timings, alcohol consumption, and late-night physical activities are the main environmental factors contributing to circadian misalignment [209]. Genetic mutations in circadian clock genes and neurodevelopmental genetic differences further disrupt circadian function, increasing the risk of various diseases, including cancer [12,14,205,210,211]. Aging, obesity, hyperglycemia, and inflammatory states can exacerbate the dysregulation of circadian rhythmicity, augmenting cancer risk [212–214].

4.1. Circadian Regulation of ECVs

Multiple lines of evidence support the circadian clock's influence on intercellular communication via ECVs, particularly exosomes. Rhythmic gene expression and protein abundance indirectly affect the temporal-specific loading of proteins in small ECVs, while the circadian clock directly governs the abundance of targeted cargo proteins in exosomes through the controlled expression of sorting proteins in the endosomal pathway. Studies have shown that ECV quantity exhibits time-dependent changes, indicating circadian variation [215], while the circadian clock directly influences the cargo content of exosomes [200]. Additionally, disruptions in circadian rhythms, such as those induced by night shift work, can alter the exosomal cargo, impacting metabolic health [216]. Plasma ECVs display dynamic changes in size distribution throughout the day, with implications for intercellular communication [217]. Overall, the circadian regulation of ECV characteristics, including size, concentration, and cargo composition, underscores the critical influence of the circadian clock in ECV biology for diagnostics and therapeutics. These rhythmic changes, governed by the circadian clock, have broad implications for intercellular communication and physiological processes, including those relevant to cancer. Understanding the role of the circadian clock in shaping ECV behavior in cancer contexts is crucial for developing time-sensitive strategies to diagnose, monitor, and treat various malignancies, including HCC, by optimizing treatment efficacy and patient outcomes. The impact of circadian rhythms on exosome secretion also suggests that aligning therapeutic interventions with these natural cycles could enhance treatment effectiveness and reduce adverse effects [8,200].

Table 4 summarizes studies on circadian influence on ECVs, showing rhythmic variations in size, concentration, and cargo composition.

Table 4. Examples of circadian regulation of ECVs.

Example	Finding	Reference
Circadian Variation in ECV Quantity	ECV quantity extracted from peripheral blood, bone marrow, and lungs of mice exhibited time-dependent changes	[215]
Circadian Control of Exosomal Cargo	Flot1 regulated circadian control over MMP 14 in tendon fibroblast small ECVs	[200]
Impact of Night Shift Work on ECV Cargo	Night shift work disrupted circadian rhythms and exosomal cargo, influencing metabolic health	[216]
Circadian Variations in Plasma ECV Characteristics	Plasma ECVs were larger at 10:00 compared to 22:00 in HIV patients	[217]
Influence of Exosomal Cargo on Insulin Resistance	Exosomes from obese mice or patients with type II diabetes could induce insulin resistance in lean mice	[218–220]
Circadian Normalization Factor for Small ECV Biomarkers	Biomarker TSG101 levels in urine showed a circadian correlation with small ECV excretion in healthy rats	[221]

4.2. Circadian Clock and HCC

Liver metabolism is intricately governed by circadian rhythms, adjusting to changes in feeding times and dietary patterns [222–226]. Central to this regulation are crucial circadian genes such as CRY, BMAL1, and CLOCK, which play pivotal roles in managing glucose levels and fat metabolism [227,228]. Disruptions in the liver's internal clock, whether from genetic factors or external influences like jet lag, significantly affect metabolic processes, including bile acid regulation. Studies link circadian rhythm disruptions to increased cancer risk, with abnormal gene expression being common in tumors [84,217,229]. Night shifts and rotating work schedules are associated with higher cancer incidence and were recognized as a probable carcinogen by the International Agency for Research on Cancer in 2007, which was reaffirmed in 2019 [230]. Circadian clocks regulate key cellular functions like growth, apoptosis, and DNA repair, impacting tumor behavior [14,205,231–235]. The disruption of circadian rhythms due to chronic jet lag has been linked to accelerated liver carcinogenesis, as well as increased susceptibility to non-alcoholic fatty liver disease, leading to the spontaneous development of HCC [236]. Recent findings have linked variations in circadian clock genes with survival rates and clinical outcomes in HCC patients [237]. The development of small-molecule modulators targeting the core circadian clock offers a new promising approach in cancer therapy, potentially leading to innovative treatments [238–240]. Research has consistently underscored the role of clock genes in the molecular mechanisms of HCC [241], driving focused efforts to develop therapeutic strategies that target these proteins to effectively treat HCC [241–244].

4.3. The Impact of the Circadian Clock on Cancer Progression via ECVs

Melatonin, a key signal of darkness, plays a vital role in regulating sleep–wake cycles and shows promise in treating conditions such as sleep disorders and jet lag, as well as enhancing cancer therapies by improving the effects of chemotherapy [245,246]. Melatonin increases the properties of exosomes, potentially enhancing their therapeutic efficacy, such as in reducing inflammatory factors and immune evasion markers like PD-L1 in cancer therapy.

Cheng et al. observed a suppression of PD-L1 expression in macrophages co-cultured with exosomes from HCC cells treated with 0.1mM melatonin, while they observed an increase in PD-L1 expression and cytokine levels in macrophages co-cultured with untreated HCC cells. This led to the conclusion that melatonin-treated exosomes effectively reduced PD-L1 expression and cytokine secretion. Therefore, manipulating circadian rhythms through exosomes could offer innovative strategies for combating inflammation and immune evasion in HCC, inhibiting STAT3 activation and potentially improving therapeutic outcomes for patients with this challenging disease [247].

In addition to the emerging evidence in HCC, there are additional data pointing to a more overarching role of circadian rhythm in cancers in general. The circadian clock regulator modulates tumorigenesis [248]. To be precise, BMAL1, an essential transcription factor regulating numerous clock target genes [249,250], promotes metastasis in colorectal cancer by enhancing exosome secretion, illustrating its crucial role in cancer progression tied to circadian regulation [17]. Research using a mouse model of nocturnal shift work showed changes in the intestinal flora and plasma ECV components affecting clock genes [251]. SIRT1, interacting with CLOCK-BMAL1, regulates PER2 and exosome secretion, impacting the tumor microenvironment and progression in breast and ovarian cancer [252–258], and is crucial in tumor dynamics and metastasis [259]. ECVs regulate key signaling pathways like GSK-3 and AMPK via non-coding RNAs, affecting the circadian regulators CRY and BMAL1/CLOCK [258,260–262]. Exosomes also modulate circadian gene expression through post-transcriptional mechanisms, playing a role in rhythmicity and offering therapeutic potential [9,263]. Additionally, exosomal miRNAs like miRNA-7239-3p from microglia influence circadian genes, impacting conditions such as glioma progression [264]. An interesting proteomic study on medulloblastoma-derived exosomes detected that the presence of proteins influenced by the circadian clock, such as transcription factors like HNF4A, can impact pathways associated with various malignancies. HNF4A, a circadian-

regulated transcription factor, modulates metabolic processes and adjusts tissue-specific circadian networks by transrepressing CLOCK-BMAL1 [244,265,266]. In HCC, the fetal variant of P2-HNF4α, typically upregulated, significantly alters the expression of BMAL1, influencing cancer progression dynamics. The presence of these proteins in exosomes suggests that circadian-influenced exosomal proteins could serve as non-invasive biomarkers for early cancer detection. Table 5 summarizes a structured overview of selected circadian biology that intersects with cancer progression through the lens of ECV dynamics.

Table 5. Impact of circadian clock components on cancer progression via ECVs.

Impact of Circadian Clock	Description	Associated Conditions/Models	Reference
Circadian Component and Cancer Role			
BMAL1 Function	BMAL1 promotes metastasis in colorectal cancer through increased exosome secretion	Colorectal cancer The link between circadian control and tumor progression	[17]
SIRT1 Mechanisms	SIRT1 interacts with CLOCK-BMAL1 to modulate PER2 stability, affecting exosome secretion and tumor environment interactions	Facilitates tumor microenvironment degradation Breast cancer	[252–255]
SIRT1 Mechanisms	SIRT1 loss leads to enhanced exosome secretion, impacting breast cancer and diabetic nephropathy	Diabetic nephropathy	[256–258]
SIRT1 in Cancer	Shift-work-related miRNA-22-3p uptake by nurses is linked to increased insulin resistance, highlighting its potential as a biomarker for diabetes prevention	Ovarian cancer	[259]
Shift Work and its Effects			
Shift-Work-Induced Changes	Chronic shift work in mice alters intestinal flora and increases colonic permeability, affecting circadian gene expression via changes in plasma ECV components	Mouse model of chronic shift work	[251]
Night Shift Exosomal Impact	Night shift conditions lead to reduced insulin sensitivity in adipocytes through alterations in exosome content, affecting core clock genes	Simulated shift work study Affects core clock genes and metabolic functions	[216]
Shift Work and Diabetes Risk	Shift-work-related miRNA-22-3p uptake by nurses is linked to increased insulin resistance, highlighting its potential as a biomarker for diabetes prevention	Shift nurses	[267]
Exosomal miRNAs and Circadian Genes	miRNA-3614-5p as a messenger of circadian misalignment in night shift workers	Contributes to metabolic dysfunction	[216]
Circadian Genes and miRNA	Circulating miRNAs like miRNA-219, miRNA-152, miRNA-494, and miRNA-142-3p regulate clock genes	Affects BMAL1 and PER1 in circadian regulation	[268–272]
Circadian Genes and miRNA	Exosomal miRNAs modulate peripheral circadian oscillators in various disease models	Has impact on glioma progression and Parkinson's disease	[9,264]
Melatonin's Therapeutic Role			
Melatonin-Enhanced Exosomes	Melatonin pre-treatment enhances anticancer and anti-inflammatory properties of exosomes, improving their therapeutic effectiveness in various clinical settings	Cancer therapies, various models	[273–275]
Anti-Inflammatory Effects	Melatonin-treated exosomes significantly reduce inflammatory markers and PD-L1 expression in macrophages, aiding in cancer therapy and reducing immune evasion	Hepatocellular carcinoma	[247]

5. Conclusions

This review highlights the significant interplay between ECVs, especially exosomes, and the development and progression of HCC, as well as the potential clinical roles of ECVs in detection and treatment. Disruptions in circadian rhythms may alter ECV function, potentially initiating or exacerbating HCC. These vesicles facilitate crucial intercellular

communication and may modify circadian rhythms in recipient cells, influencing disease progression.

Exosomal cargo-sorting mechanisms in HCC involve RNA-binding proteins, Rab GTPases, and post-translational modifications. For example, the overexpression of VpsA4, an ATPase essential to the ESCRT pathway, modifies exosomal miRNA levels in HCC cells [276]. Rab GTPases and RNA-binding proteins are also critical in exosome biogenesis and influence cell signaling and tumor progression [277]. Moreover, exosomal lipids are also essential in exosome biogenesis and function [278]. Further research into the sorting mechanisms of exosomal cargo is crucial. Understanding the roles of RNA-binding proteins, Rab GTPases, and post-translational modifications will enhance our comprehension of how exosomes influence disease processes. Additionally, the effect of circadian clock genes on the tumor microenvironment, particularly regarding immune cell infiltration and overall cancer progression, underscores the importance of exploring how circadian genes impact exosome-mediated communication.

ECVs facilitate intercellular communication through their diverse cargo. Quantitative proteomic analyses across various cell lines, including hepatocytes, have identified unique ECV protein profiles that can predict small changes in serum [279]. In addition, exosomal biomarkers, such as miRNAs and circRNAs, showed higher sensitivity and specificity than AFP, a traditional biomarker widely used in clinical practice for detecting HCC [34,51]. To date, several methods for exosome isolation have been developed to enhance precision and throughput, including microfluidics-based isolation, density gradient ultracentrifugation, immunoaffinity capture, size-exclusion chromatography, polymer-based precipitation, ultrafiltration, field-flow fractionation, and differential ultracentrifugation. Immunoaffinity capture techniques use antibodies targeting specific exosome markers, enabling selective isolation from specific cell types like hepatocytes [280,281].

Exosomal biomarkers are stable in bodily fluids and offer non-invasive detection methods, providing detailed insights into tumor dynamics, including growth, metastasis, and treatment responses [24]. However, despite these advantages, the clinical implementation of exosomal biomarkers faces several challenges. The heterogeneity of exosomes, derived from various cell types, leads to variability in their content and function, complicating the isolation of tumor-specific exosomes [56,282]. Current isolation methods, such as ultracentrifugation and immunoaffinity capture, vary in efficiency and specificity, underscoring the need for standardized protocols to ensure reproducibility and reliability in clinical settings [281]. Accurate quantification and characterization of exosomal biomarkers are challenging due to their small size and complex cargo, necessitating the development of more precise and sensitive analytical technologies [52]. Furthermore, extensive clinical trials and validation studies are required to establish the efficacy of exosomal biomarkers in routine clinical practice. Regulatory approval for new diagnostic tools based on exosomal biomarkers involves rigorous evaluation and adherence to standards [52,283]. Addressing these challenges can highlight the potential advantages of exosomal biomarkers and support their successful clinical implementation in HCC diagnosis and treatment [284].

Innovative techniques for non-invasive biomarkers, such as isolating cell-specific exosomes, could offer promising avenues for early disease detection and intervention. One study showed that employing ASGR1 to purify exosomes derived from hepatocytes improved the detection of liver disease biomarkers [154]. To further refine this technique, focusing on additional liver-specific proteins such as hepatocyte-specific antigens, liver-specific transport proteins, and liver fatty-acid-binding proteins could significantly enhance the precision and efficacy of these diagnostic tools.

Furthermore, aligning medical treatments with the patient's body clock through chronotherapy or pharmacological manipulations of the circadian clock could retard tumor growth [205,285]. Ideally, insights from the circadian regulation of exosome release and composition should be considered to optimize treatment efficacy. Therefore, the time of blood sample collection should be considered covariable in the design of clinical studies.

This parameter may promote insights into how the circadian clock influences the biology of cancer via exosomal proteins, which are crucial mediators in intercellular communication.

Understanding the complex interplay among exosomal components, cancer biology, and the circadian rhythm is essential. This relationship forms the basis of a rich field of study that promises to broaden our understanding of the mechanisms underlying malignancy and innovate diagnostic and therapeutic strategies. The ultimate goal is to leverage this knowledge to develop more personalized, timely, and effective treatment approaches, improving clinical outcomes for patients with HCC.

Future research should integrate these findings into clinical practice to enhance the precision and efficacy of HCC treatments. Leveraging ECVs for early disease detection, targeted drug delivery, and immune modulation in HCC provides significant advantages, including higher sensitivity and specificity in diagnostics, improved therapeutic outcomes, and reduced systemic toxicity compared to existing methods. However, addressing challenges such as exosome heterogeneity, isolation, and standardization is critical. Developing standardized protocols for exosome isolation and characterization, elucidating the mechanisms of ECV biogenesis and cargo sorting, and conducting comprehensive clinical trials are essential steps to validate the clinical utility of exosome-based therapies.

Author Contributions: All authors contributed their intellectual input, discussed the information, and made improvements to the manuscript. Conceptualization, B.F.; writing—original draft preparation, L.U.; writing—review and editing; L.G. and N.F.E., writing, supervising, editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Willms, E.; Johansson, H.J.; Mäger, I.; Lee, Y.; Blomberg, K.E.M.; Sadik, M.; Alaarg, A.; Smith, C.E.; Lehtiö, J.; EL Andaloussi, S.; et al. Cells release subpopulations of exosomes with distinct molecular and biological properties. *Sci. Rep.* **2016**, *6*, 22519. [CrossRef]
2. Dhar, R.; Gorai, S.; Devi, A.; Muthusamy, R.; Alexiou, A.; Papadakis, M. Decoding of exosome heterogeneity for cancer theranostics. *Clin. Transl. Med.* **2023**, *13*, e1288. [CrossRef]
3. Almeria, C.; Kreß, S.; Weber, V.; Egger, D.; Kasper, C. Heterogeneity of mesenchymal stem cell-derived extracellular vesicles is highly impacted by the tissue/cell source and culture conditions. *Cell Biosci.* **2022**, *12*, 51. [CrossRef]
4. Ha, D.; Yang, N.; Nadithe, V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: Current perspectives and future challenges. *Acta Pharm. Sin. B* **2016**, *6*, 287–296. [CrossRef]
5. Zhu, L.; Sun, H.-T.; Wang, S.; Huang, S.-L.; Zheng, Y.; Wang, C.-Q.; Hu, B.-Y.; Qin, W.; Zou, T.-T.; Fu, Y.; et al. Isolation and characterization of exosomes for cancer research. *J. Hematol. Oncol.* **2020**, *13*, 152. [CrossRef]
6. Huda, M.N.; Nafiujjaman, M.; Deaguero, I.G.; Okonkwo, J.; Hill, M.L.; Kim, T.; Nurunnabi, M. Potential Use of Exosomes as Diagnostic Biomarkers and in Targeted Drug Delivery: Progress in Clinical and Preclinical Applications. *ACS Biomater. Sci. Eng.* **2021**, *7*, 2106–2149. [CrossRef]
7. Kojima, S.; Shingle, D.L.; Green, C.B. Post-transcriptional control of circadian rhythms. *J. Cell Sci.* **2011**, *124 Pt 3*, 311–320. [CrossRef]
8. Guo, J.; Cheng, P.; Yuan, H.; Liu, Y. The Exosome Regulates Circadian Gene Expression in a Posttranscriptional Negative Feedback Loop. *Cell* **2009**, *138*, 1236–1246. [CrossRef] [PubMed]
9. Li, Z.; Li, Y.; Xu, X.; Gu, J.; Chen, H.; Gui, Y. Exosomes rich in Wnt5 improved circadian rhythm dysfunction via enhanced PPARγ activity in the 6-hydroxydopamine model of Parkinson's disease. *Neurosci. Lett.* **2023**, *802*, 137139. [CrossRef] [PubMed]
10. Hahn, K.; Sundar, I.K. Current Perspective on the Role of the Circadian Clock and Extracellular Matrix in Chronic Lung Diseases. *Int. J. Environ. Res. Public Health* **2023**, *20*, 2455. [CrossRef]
11. Chen, W.-H.; Huang, Q.-Y.; Wang, Z.-Y.; Zhuang, X.-X.; Lin, S.; Shi, Q.-Y. Therapeutic potential of exosomes/miRNAs in polycystic ovary syndrome induced by the alteration of circadian rhythms. *Front. Endocrinol.* **2022**, *13*, 918805. [CrossRef]
12. Sulli, G.; Lam, M.T.Y.; Panda, S. Interplay between Circadian Clock and Cancer: New Frontiers for Cancer Treatment. *Trends Cancer* **2019**, *5*, 475–494. [CrossRef]

13. Altman, B.J.; Hsieh, A.L.; Sengupta, A.; Krishnanaiah, S.Y.; Stine, Z.E.; Walton, Z.E.; Gouw, A.M.; Venkataraman, A.; Li, B.; Goraksha-Hicks, P.; et al. MYC Disrupts the Circadian Clock and Metabolism in Cancer Cells. *Cell Metab.* **2015**, *22*, 1009–1019. [CrossRef]
14. Fekry, B.; Eckel-Mahan, K. The circadian clock and cancer: Links between circadian disruption and disease Pathology. *J. Biochem.* **2022**, *171*, 477–486. [CrossRef]
15. Verlande, A.; Masri, S. Circadian Clocks and Cancer: Timekeeping Governs Cellular Metabolism. *Trends Endocrinol. Metab.* **2019**, *30*, 445–458. [CrossRef]
16. Fekry, B.; Ribas-Latre, A.; Baumgartner, C.; Mohamed, A.M.T.; Kolonin, M.G.; Sladek, F.M.; Younes, M.; Eckel-Mahan, K.L. HNF4α-Deficient Fatty Liver Provides a Permissive Environment for Sex-Independent Hepatocellular Carcinoma. *Cancer Res.* **2019**, *79*, 5860–5873. [CrossRef]
17. Dong, P.; Wang, Y.; Liu, Y.; Zhu, C.; Lin, J.; Qian, R.; Hua, L.; Lu, C. BMAL1 induces colorectal cancer metastasis by stimulating exosome secretion. *Mol. Biol. Rep.* **2022**, *49*, 373–384. [CrossRef]
18. Ortega-Campos, S.M.; Verdugo-Sivianes, E.M.; Amiama-Roig, A.; Blanco, J.R.; Carnero, A. Interactions of circadian clock genes with the hallmarks of cancer. *Biochim. Biophys. Acta BBA Rev. Cancer* **2023**, *1878*, 188900. [CrossRef]
19. Duffy, M.J. Tumor markers in clinical practice: A review focusing on common solid cancers. *Med. Princ. Pr.* **2012**, *22*, 4–11. [CrossRef]
20. Locker, G.Y.; Hamilton, S.; Harris, J.; Jessup, J.M.; Kemeny, N.; Macdonald, J.S.; Somerfield, M.R.; Hayes, D.F.; Bast, R.C., Jr. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J. Clin. Oncol.* **2006**, *24*, 5313–5327. [CrossRef]
21. He, C.-Z.; Zhang, K.-H.; Li, Q.; Liu, X.-H.; Hong, Y.; Lv, N.-H. Combined use of AFP, CEA, CA125 and CAl9-9 improves the sensitivity for the diagnosis of gastric cancer. *BMC Gastroenterol.* **2013**, *13*, 87. [CrossRef]
22. Gupta, D.; Lis, C.G. Role of CA125 in predicting ovarian cancer survival—A review of the epidemiological literature. *J. Ovarian Res.* **2009**, *2*, 13. [CrossRef]
23. Wong, C.H.; Chen, Y.C. Clinical significance of exosomes as potential biomarkers in cancer. *World J. Clin. Cases* **2019**, *7*, 171–190. [CrossRef]
24. Kalluri, R.; LeBleu, V.S. The biology, function, and biomedical applications of exosomes. *Science* **2020**, *367*, eaau6977. [CrossRef]
25. Krishnan, A.; Bhattacharya, B.; Mandal, D.; Dhar, R.; Muthu, S. Salivary exosomes: A theranostics secret of oral cancer—Correspondence. *Int. J. Surg.* **2022**, *108*, 106990. [CrossRef]
26. Wang, X.; Tian, L.; Lu, J.; Ng, I.O. Exosomes and cancer—Diagnostic and prognostic biomarkers and therapeutic vehicle. *Oncogenesis* **2022**, *11*, 54. [CrossRef]
27. Makler, A.; Asghar, W. Exosomal biomarkers for cancer diagnosis and patient monitoring. *Expert. Rev. Mol. Diagn.* **2020**, *20*, 387–400. [CrossRef]
28. Hanjani, N.A.; Esmaelizad, N.; Zanganeh, S.; Gharavi, A.T.; Heidarizadeh, P.; Radfar, M.; Omidi, F.; MacLoughlin, R.; Doroudian, M. Emerging role of exosomes as biomarkers in cancer treatment and diagnosis. *Crit. Rev. Oncol. Hematol.* **2022**, *169*, 103565. [CrossRef]
29. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2018**, *68*, 394–424. [CrossRef]
30. Anwanwan, D.; Singh, S.K.; Singh, S.; Saikam, V.; Singh, R. Challenges in liver cancer and possible treatment approaches. *Biochim. Biophys. Acta BBA Rev. Cancer* **2020**, *1873*, 188314. [CrossRef]
31. National Comprehensive Cancer Nwtwork. *NCCN Clinical Practice Guidelines in Oncology: Hepatobiliary Cancers*; Version 1.2023; National Comprehensive Cancer Network: Plymouth Meeting, PA, USA, 2023; Available online: https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1438.2023 (accessed on 10 June 2024).
32. Singal, A.G.; Llovet, J.M.; Yarchoan, M.; Mehta, N.; Heimbach, J.K.; Dawson, L.A.; Jou, J.H.; Kulik, L.M.; Agopian, V.G.; Marrero, J.A.; et al. AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. *Hepatology* **2023**, *78*, 1922–1965. [CrossRef]
33. Choi, D.T.; Kum, H.C.; Park, S.; Ohsfeldt, R.L.; Shen, Y.; Parikh, N.D.; Singal, A.G. Hepatocellular Carcinoma Screening Is Associated with Increased Survival of Patients with Cirrhosis. *Clin. Gastroenterol. Hepatol.* **2019**, *17*, 976–987.e4. [CrossRef]
34. Yang, J.D.; Hainaut, P.; Gores, G.J.; Amadou, A.; Plymoth, A.; Roberts, L.R. A global view of hepatocellular carcinoma: Trends, risk, prevention and management. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 589–604. [CrossRef]
35. Lok, A.S.; Sterling, R.K.; Everhart, J.E.; Wright, E.C.; Hoefs, J.C.; Di Bisceglie, A.M.; Morgan, T.R.; Kim, H.; Lee, W.M.; Bonkovsky, H.L.; et al. Des-γ-carboxy prothrombin and α-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* **2010**, *138*, 493–502. [CrossRef]
36. Pinyol, R.; Montal, R.; Bassaganyas, L.; Sia, D.; Takayama, T.; Chau, G.-Y.; Mazzaferro, V.; Roayaie, S.; Lee, H.C.; Kokudo, N.; et al. Molecular predictors of prevention of recurrence in HCC with sorafenib as adjuvant treatment and prognostic factors in the phase 3 STORM trial. *Gut* **2019**, *68*, 1065–1075. [CrossRef]
37. Parikh, N.D.; Mehta, A.S.; Singal, A.G.; Block, T.; Marrero, J.A.; Lok, A.S. Biomarkers for the Early Detection of Hepatocellular Carcinoma. *Cancer Epidemiol. Biomark. Prev.* **2020**, *29*, 2495–2503. [CrossRef]
38. Chen, W.; Mao, Y.; Liu, C.; Wu, H.; Chen, S. Exosome in Hepatocellular Carcinoma: An update. *J. Cancer* **2021**, *12*, 2526–2536. [CrossRef]

39. Szabo, G.; Momen-Heravi, F. Extracellular vesicles in liver disease and potential as biomarkers and therapeutic targets. *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 455–466. [CrossRef]
40. Théry, C.; Zitvogel, L.; Amigorena, S. Exosomes: Composition, biogenesis and function. *Nat. Rev. Immunol.* **2002**, *2*, 569–579. [CrossRef]
41. Edwards, K.A.; Leete, J.J.; Smith, E.G.; Quick, A.; Modica, C.M.; Wassermann, E.M.; Polejaeva, E.; Dell, K.C.; LoPresti, M.; Walker, P.; et al. Elevations in Tumor Necrosis Factor Alpha and Interleukin 6 from Neuronal-Derived Extracellular Vesicles in Repeated Low-Level Blast Exposed Personnel. *Front. Neurol.* **2022**, *13*, 723923. [CrossRef]
42. Raposo, G.; Stoorvogel, W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J. Cell Biol.* **2013**, *200*, 373–383. [CrossRef]
43. Sheta, M.; Taha, E.A.; Lu, Y.; Eguchi, T. Extracellular Vesicles: New Classification and Tumor Immunosuppression. *Biology* **2023**, *12*, 110. [CrossRef]
44. Pan, B.T.; Johnstone, R.M. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: Selective externalization of the receptor. *Cell* **1983**, *33*, 967–978. [CrossRef]
45. Gurung, S.; Perocheau, D.; Touramanidou, L.; Baruteau, J. The exosome journey: From biogenesis to uptake and intracellular signalling. *Cell Commun. Signal.* **2021**, *19*, 47. [CrossRef]
46. Mulcahy, L.A.; Pink, R.C.; Carter, D.R. Routes and mechanisms of extracellular vesicle uptake. *J. Extracell. Vesicles* **2014**, *3*, 24641. [CrossRef]
47. Mathieu, M.; Martin-Jaular, L.; Lavieu, G.; Théry, C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat. Cell Biol.* **2019**, *21*, 9–17. [CrossRef]
48. Paskeh, M.D.A.; Entezari, M.; Mirzaei, S.; Zabolian, A.; Saleki, H.; Naghdi, M.J.; Sabet, S.; Khoshbakht, M.A.; Hashemi, M.; Hushmandi, K.; et al. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. *J. Hematol. Oncol.* **2022**, *15*, 83. [CrossRef]
49. Colombo, M.; Raposo, G.; Théry, C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 255–289. [CrossRef]
50. Lötvall, J.; Hill, A.F.; Hochberg, F.; Buzás, E.I.; Di Vizio, D.; Gardiner, C.; Gho, Y.S.; Kurochkin, I.V.; Mathivanan, S.; Quesenberry, P.; et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: A position statement from the International Society for Extracellular Vesicles. *J. Extracell. Vesicles* **2014**, *3*, 26913. [CrossRef]
51. Wang, X.; Huang, J.; Chen, W.; Li, G.; Li, Z.; Lei, J. The updated role of exosomal proteins in the diagnosis, prognosis, and treatment of cancer. *Exp. Mol. Med.* **2022**, *54*, 1390–1400. [CrossRef]
52. Smolarz, M.; Pietrowska, M.; Matysiak, N.; Mielańczyk, Ł.; Widłak, P. Proteome profiling of exosomes purified from a small amount of human serum: The problem of co-purified serum components. *Proteomes* **2019**, *7*, 18. [CrossRef]
53. Liang, Y.; Lehrich, B.M.; Zheng, S.; Lu, M. Emerging methods in biomarker identification for extracellular vesicle-based liquid biopsy. *J. Extracell. Vesicles* **2021**, *10*, e12090. [CrossRef]
54. Wang, N.; Song, X.; Liu, L.; Niu, L.; Wang, X.; Song, X.; Xie, L. Circulating exosomes contain protein biomarkers of metastatic non-small-cell lung cancer. *Cancer Sci.* **2018**, *109*, 1701–1709. [CrossRef]
55. Mazurov, D.; Barbashova, L.; Filatov, A. Tetraspanin protein CD 9 interacts with metalloprotease CD 10 and enhances its release via exosomes. *FEBS J.* **2013**, *280*, 1200–1213. [CrossRef]
56. Malla, R.R.; Pandrangi, S.; Kumari, S.; Gavara, M.M.; Badana, A.K. Exosomal tetraspanins as regulators of cancer progression and metastasis and novel diagnostic markers. *Asia Pac. J. Clin. Oncol.* **2018**, *14*, 383–391. [CrossRef]
57. Mashouri, L.; Yousefi, H.; Aref, A.R.; Ahadi, A.M.; Molaei, F.; Alahari, S.K. Exosomes: Composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol. Cancer* **2019**, *18*, 75. [CrossRef]
58. Nolte-'t Hoen, E.N.; Buermans, H.P.; Waasdorp, M.; Stoorvogel, W.; Wauben, M.H.; 't Hoen, P.A. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. *Nucleic Acids Res.* **2012**, *40*, 9272–9285. [CrossRef]
59. Doyle, L.M.; Wang, M.Z. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* **2019**, *8*, 727. [CrossRef]
60. Zhang, Y.; Liu, Y.; Liu, H.; Tang, W.H. Exosomes: Biogenesis, biologic function and clinical potential. *Cell Biosci.* **2019**, *9*, 19. [CrossRef]
61. Ge, L.; Zhang, N.; Li, D.; Wu, Y.; Wang, H.; Wang, J. Circulating exosomal small RNAs are promising non-invasive diagnostic biomarkers for gastric cancer. *J. Cell Mol. Med.* **2020**, *24*, 14502–14513. [CrossRef]
62. Li, X.; Chen, R.; Kemper, S.; Brigstock, D.R. Dynamic changes in function and proteomic composition of extracellular vesicles from hepatic stellate cells during cellular activation. *Cells* **2020**, *9*, 290. [CrossRef]
63. Malhi, H. Emerging role of extracellular vesicles in liver diseases. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2019**, *317*, G739–G749. [CrossRef]
64. Kostallari, E.; Hirsova, P.; Prasnicka, A.; Verma, V.K.; Yaqoob, U.; Wongjarupong, N.; Roberts, L.R.; Shah, V.H. Hepatic stellate cell–derived platelet-derived growth factor receptor-alpha-enriched extracellular vesicles promote liver fibrosis in mice through SHP2. *Hepatology* **2018**, *68*, 333–348. [CrossRef]
65. Dasgupta, D.; Nakao, Y.; Mauer, A.S.; Thompson, J.M.; Sehrawat, T.S.; Liao, C.-Y.; Krishnan, A.; Lucien, F.; Guo, Q.; Liu, M.; et al. IRE1A stimulates hepatocyte-derived extracellular vesicles that promote inflammation in mice with steatohepatitis. *Gastroenterology* **2020**, *159*, 1487–1503.e17. [CrossRef]

66. Kalluri, R. The biology and function of exosomes in cancer. *J. Clin. Investig.* **2016**, *126*, 1208–1215. [CrossRef]
67. Bebelman, M.P.; Smit, M.J.; Pegtel, D.M.; Baglio, S.R. Biogenesis and function of extracellular vesicles in cancer. *Pharmacol. Ther.* **2018**, *188*, 1–11. [CrossRef]
68. Osaki, M.; Okada, F. Exosomes and their role in cancer progression. *Yonago Acta Medica* **2019**, *62*, 182–190. [CrossRef]
69. Zhong, Y.; Li, H.; Li, P.; Chen, Y.; Zhang, M.; Yuan, Z.; Zhang, Y.; Xu, Z.; Luo, G.; Fang, Y.; et al. Exosomes: A New Pathway for Cancer Drug Resistance. *Front. Oncol.* **2021**, *11*, 743556. [CrossRef]
70. Ogata-Kawata, H.; Izumiya, M.; Kurioka, D.; Honma, Y.; Yamada, Y.; Furuta, K.; Gunji, T.; Ohta, H.; Okamoto, H.; Sonoda, H.; et al. Circulating exosomal microRNAs as biomarkers of colon cancer. *PLoS ONE* **2014**, *9*, e92921. [CrossRef] [PubMed]
71. Liu, T.; Liu, D.; Guan, S.; Dong, M. Diagnostic role of circulating MiR-21 in colorectal cancer: A update meta-analysis. *Ann. Med.* **2021**, *53*, 87–102. [CrossRef]
72. Zhou, W.; Fong, M.Y.; Min, Y.; Somlo, G.; Liu, L.; Palomares, M.R.; Yu, Y.; Chow, A.; O'Connor, S.T.F.; Chin, A.R.; et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* **2014**, *25*, 501–515. [CrossRef]
73. Thind, A.; Wilson, C. Exosomal miRNAs as cancer biomarkers and therapeutic targets. *J. Extracell. Vesicles* **2016**, *5*, 31292. [CrossRef] [PubMed]
74. Eichelser, C.; Stückrath, I.; Müller, V.; Milde-Langosch, K.; Wikman, H.; Pantel, K.; Schwarzenbach, H. Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients. *Oncotarget* **2014**, *5*, 9650–9663. [CrossRef] [PubMed]
75. Que, R.; Ding, G.; Chen, J.; Cao, L. Analysis of serum exosomal microRNAs and clinicopathologic features of patients with pancreatic adenocarcinoma. *World J. Surg. Oncol.* **2013**, *11*, 219. [CrossRef] [PubMed]
76. Wang, J.; Chen, J.; Chang, P.; LeBlanc, A.; Li, D.; Abbruzzesse, J.L.; Frazier, M.L.; Killary, A.M.; Sen, S. MicroRNAs in Plasma of Pancreatic Ductal Adenocarcinoma Patients as Novel Blood-Based Biomarkers of Disease. *Cancer Prev. Res.* **2009**, *2*, 807–813. [CrossRef] [PubMed]
77. Wong, T.-S.; Liu, X.-B.; Wong, B.Y.-H.; Ng, R.W.-M.; Yuen, A.P.-W.; Wei, W.I. Mature miR-184 as Potential Oncogenic microRNA of Squamous Cell Carcinoma of Tongue. *Clin. Cancer Res.* **2008**, *14*, 2588–2592. [CrossRef]
78. Yamamoto, Y.; Kosaka, N.; Tanaka, M.; Koizumi, F.; Kanai, Y.; Mizutani, T.; Murakami, Y.; Kuroda, M.; Miyajima, A.; Kato, T.; et al. MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. *Biomarkers* **2009**, *14*, 529–538. [CrossRef] [PubMed]
79. Yang, J.; Dong, W.; Zhang, H.; Zhao, H.; Zeng, Z.; Zhang, F.; Li, Q.; Duan, X.; Hu, Y.; Xiao, W. Exosomal microRNA panel as a diagnostic biomarker in patients with hepatocellular carcinoma. *Front. Cell Dev. Biol.* **2022**, *10*, 927251. [CrossRef] [PubMed]
80. Qu, Z.; Wu, J.; Wu, J.; Ji, A.; Qiang, G.; Jiang, Y.; Jiang, C.; Ding, Y. Exosomal miR-665 as a novel minimally invasive biomarker for hepatocellular carcinoma diagnosis and prognosis. *Oncotarget* **2017**, *8*, 80666. [CrossRef]
81. Sohn, W.; Kim, J.; Kang, S.H.; Yang, S.R.; Cho, J.-Y.; Cho, H.C.; Shim, S.G.; Paik, Y.-H. Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. *Exp. Mol. Med.* **2015**, *47*, e184. [CrossRef]
82. Tomimaru, Y.; Eguchi, H.; Nagano, H.; Wada, H.; Kobayashi, S.; Marubashi, S.; Tanemura, M.; Tomokuni, A.; Takemasa, I.; Umeshita, K.; et al. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. *J. Hepatol.* **2012**, *56*, 167–175. [CrossRef]
83. Park, N.J.; Zhou, H.; Elashoff, D.; Henson, B.S.; Kastratovic, D.A.; Abemayor, E.; Wong, D.T. Salivary microRNA: Discovery, Characterization, and Clinical Utility for Oral Cancer Detection. *Clin. Cancer Res.* **2009**, *15*, 5473–5477. [CrossRef] [PubMed]
84. Zhang, P.-F.; Gao, C.; Huang, X.-Y.; Lu, J.-C.; Guo, X.-J.; Shi, G.-M.; Cai, J.-B.; Ke, A.-W. Cancer cell-derived exosomal circUHRF1 induces natural killer cell exhaustion and may cause resistance to anti-PD1 therapy in hepatocellular carcinoma. *Mol. Cancer* **2020**, *19*, 110. [CrossRef] [PubMed]
85. Zhang, P.-F.; Wei, C.-Y.; Huang, X.-Y.; Peng, R.; Yang, X.; Lu, J.-C.; Zhang, C.; Gao, C.; Cai, J.-B.; Gao, P.-T.; et al. Circular RNA circTRIM33–12 acts as the sponge of MicroRNA-191 to suppress hepatocellular carcinoma progression. *Mol. Cancer* **2019**, *18*, 1–15. [CrossRef] [PubMed]
86. Huang, X.-Y.; Huang, Z.-L.; Huang, J.; Xu, B.; Huang, X.-Y.; Xu, Y.-H.; Zhou, J.; Tang, Z.-Y. Exosomal circRNA-100338 promotes hepatocellular carcinoma metastasis via enhancing invasiveness and angiogenesis. *J. Exp. Clin. Cancer Res.* **2020**, *39*, 20. [CrossRef]
87. Chen, W.; Quan, Y.; Fan, S.; Wang, H.; Liang, J.; Huang, L.; Chen, L.; Liu, Q.; He, P.; Ye, Y. Exosome-transmitted circular RNA hsa_circ_0051443 suppresses hepatocellular carcinoma progression. *Cancer Lett.* **2020**, *475*, 119–128. [CrossRef]
88. Xian, J.; Su, W.; Liu, L.; Rao, B.; Lin, M.; Feng, Y.; Qiu, F.; Chen, J.; Zhou, Q.; Zhao, Z.; et al. Identification of three circular RNA cargoes in serum exosomes as diagnostic biomarkers of non–small-cell lung cancer in the Chinese population. *J. Mol. Diagn.* **2020**, *22*, 1096–1108. [CrossRef]
89. Kang, Y.; You, J.; Gan, Y.; Chen, Q.; Huang, C.; Chen, F.; Xu, X.; Chen, L. Serum and serum exosomal CircRNAs hsa_circ_0001492, hsa_circ_0001439, and hsa_circ_0000896 as diagnostic biomarkers for lung adenocarcinoma. *Front. Oncol.* **2022**, *12*, 912246. [CrossRef]
90. Luo, Y.; Ma, J.; Liu, F.; Guo, J.; Gui, R. Diagnostic value of exosomal circMYC in radioresistant nasopharyngeal carcinoma. *Head. Neck* **2020**, *42*, 3702–3711. [CrossRef]
91. Xia, D.; Gu, X. Plasmatic exosome-derived circRNAs panel act as fingerprint for glioblastoma. *Aging* **2021**, *13*, 19575–19586. [CrossRef]

92. He, Y.-D.; Tao, W.; He, T.; Wang, B.-Y.; Tang, X.-M.; Zhang, L.-M.; Wu, Z.-Q.; Deng, W.-M.; Zhang, L.-X.; Shao, C.-K.; et al. A urine extracellular vesicle circRNA classifier for detection of high-grade prostate cancer in patients with prostate-specific antigen 2–10 ng/mL at initial biopsy. *Mol. Cancer* **2021**, *20*, 96. [CrossRef] [PubMed]
93. Royo, F.; Moreno, L.; Mleczko, J.; Palomo, L.; Gonzalez, E.; Cabrera, D.; Cogolludo, A.; Vizcaino, F.P.; Van-Liempd, S.; Falcon-Perez, J.M. Hepatocyte-secreted extracellular vesicles modify blood metabolome and endothelial function by an arginase-dependent mechanism. *Sci. Rep.* **2017**, *7*, 42798. [CrossRef] [PubMed]
94. Nojima, H.; Freeman, C.M.; Schuster, R.M.; Japtok, L.; Kleuser, B.; Edwards, M.J.; Gulbins, E.; Lentsch, A.B. Hepatocyte exosomes mediate liver repair and regeneration via sphingosine-1-phosphate. *J. Hepatol.* **2016**, *64*, 60–68. [CrossRef] [PubMed]
95. Wang, R.; Ding, Q.; Yaqoob, U.; de Assuncao, T.M.; Verma, V.K.; Hirsova, P.; Cao, S.; Mukhopadhyay, D.; Huebert, R.C.; Shah, V.H. Exosome adherence and internalization by hepatic stellate cells triggers sphingosine 1-phosphate-dependent migration. *J. Biol. Chem.* **2015**, *290*, 30684–30696. [CrossRef] [PubMed]
96. Chen, L.; Charrier, A.; Zhou, Y.; Chen, R.; Yu, B.; Agarwal, K.; Tsukamoto, H.; Lee, L.J.; Paulaitis, M.E.; Brigstock, D.R. Epigenetic regulation of connective tissue growth factor by MicroRNA-214 delivery in exosomes from mouse or human hepatic stellate cells. *Hepatology* **2014**, *59*, 1118–1129. [CrossRef] [PubMed]
97. Chen, L.; Chen, R.; Velazquez, V.M.; Brigstock, D.R. Fibrogenic signaling is suppressed in hepatic stellate cells through targeting of connective tissue growth factor (CCN2) by cellular or exosomal microRNA-199a-5p. *Am. J. Pathol.* **2016**, *186*, 2921–2933. [CrossRef] [PubMed]
98. Zhang, X.; Chen, F.; Huang, P.; Wang, X.; Zhou, K.; Zhou, C.; Yu, L.; Peng, Y.; Fan, J.; Zhou, J.; et al. Exosome-depleted MiR-148a-3p derived from Hepatic Stellate Cells Promotes Tumor Progression via ITGA5/PI3K/Akt Axis in Hepatocellular Carcinoma. *Int. J. Biol. Sci.* **2022**, *18*, 2249–2260. [CrossRef] [PubMed]
99. Li, X.; Liu, R.; Huang, Z.; Gurley, E.C.; Wang, X.; Wang, J.; He, H.; Yang, H.; Lai, G.; Zhang, L.; et al. Cholangiocyte-derived exosomal long noncoding RNA H19 promotes cholestatic liver injury in mouse and humans. *Hepatology* **2018**, *68*, 599–615. [CrossRef] [PubMed]
100. Witek, R.P.; Yang, L.; Liu, R.; Jung, Y.; Omenetti, A.; Syn, W.K.; Choi, S.S.; Cheong, Y.; Fearing, C.M.; Agboola, K.M.; et al. Liver cell–derived microparticles activate hedgehog signaling and alter gene expression in hepatic endothelial cells. *Gastroenterology* **2009**, *136*, 320–330.e2. [CrossRef] [PubMed]
101. Newman, L.A.; Muller, K.; Rowland, A. Circulating cell-specific extracellular vesicles as biomarkers for the diagnosis and monitoring of chronic liver diseases. *Cell Mol. Life Sci.* **2022**, *79*, 232. [CrossRef]
102. Sung, S.; Kim, J.; Jung, Y. Liver-derived exosomes and their implications in liver pathobiology. *Int. J. Mol. Sci.* **2018**, *19*, 3715. [CrossRef] [PubMed]
103. Wang, X.; Rao, H.; Liu, F.; Wei, L.; Li, H.; Wu, C. Recent Advances in Adipose Tissue Dysfunction and Its Role in the Pathogenesis of Non-Alcoholic Fatty Liver Disease. *Cells* **2021**, *10*, 3300. [CrossRef] [PubMed]
104. Pan, X.; Zhang, Y. Hepatocyte nuclear factor 4α in the pathogenesis of non-alcoholic fatty liver disease. *Chin. Med. J.* **2022**, *135*, 1172–1181. [CrossRef] [PubMed]
105. Eguchi, A.; Yan, R.; Pan, S.Q.; Wu, R.; Kim, J.; Chen, Y.; Ansong, C.; Smith, R.D.; Tempaku, M.; Ohno-Machado, L.; et al. Comprehensive characterization of hepatocyte-derived extracellular vesicles identifies direct miRNA-based regulation of hepatic stellate cells and DAMP-based hepatic macrophage IL-1β and IL-17 upregulation in alcoholic hepatitis mice. *J. Mol. Med.* **2020**, *98*, 1021–1034. [CrossRef] [PubMed]
106. Keinicke, H.; Sun, G.; Mentzel, C.M.J.; Fredholm, M.; John, L.M.; Andersen, B.; Raun, K.; Kjaergaard, M. FGF21 regulates hepatic metabolic pathways to improve steatosis and inflammation. *Endocr. Connect.* **2020**, *9*, 755–768. [CrossRef]
107. Julich-Haertel, H.; Urban, S.K.; Krawczyk, M.; Willms, A.; Jankowski, K.; Patkowski, W.; Kruk, B.; Krasnodębski, M.; Ligocka, J.; Schwab, R.; et al. Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma. *J. Hepatol.* **2017**, *67*, 282–292. [CrossRef] [PubMed]
108. Arbelaiz, A.; Azkargorta, M.; Krawczyk, M.; Santos-Laso, A.; Lapitz, A.; Perugorria, M.J.; Erice, O.; Gonzalez, E.; Jimenez-Agüero, R.; La Casta, A.; et al. Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. *Hepatology* **2017**, *66*, 1125–1143. [CrossRef]
109. Chen, L.; Guo, P.; He, Y.; Chen, Z.; Chen, L.; Luo, Y.; Qi, L.; Liu, Y.; Wu, Q.; Cui, Y.; et al. HCC-derived exosomes elicit HCC progression and recurrence by epithelial-mesenchymal transition through MAPK/ERK signalling pathway. *Cell Death Dis.* **2018**, *9*, 513. [CrossRef] [PubMed]
110. Cao, L.-q.; Yang, X.-w.; Chen, Y.-b.; Zhang, D.-w.; Jiang, X.-F.; Xue, P. Exosomal miR-21 regulates the TETs/PTENp1/PTEN pathway to promote hepatocellular carcinoma growth. *Mol. Cancer* **2019**, *18*, 1–14. [CrossRef]
111. Xue, X.; Wang, X.; Zhao, Y.; Hu, R.; Qin, L. Exosomal miR-93 promotes proliferation and invasion in hepatocellular carcinoma by directly inhibiting TIMP2/TP53INP1/CDKN1A. *Biochem. Biophys. Res. Commun.* **2018**, *502*, 515–521. [CrossRef]
112. Verma, V.K.; Li, H.; Wang, R.; Hirsova, P.; Mushref, M.; Liu, Y.; Cao, S.; Contreras, P.C.; Malhi, H.; Kamath, P.S.; et al. Alcohol stimulates macrophage activation through caspase-dependent hepatocyte derived release of CD40L containing extracellular vesicles. *J. Hepatol.* **2016**, *64*, 651–660. [CrossRef]
113. Liu, D.; Kang, H.; Gao, M.; Jin, L.; Zhang, F.; Chen, D.; Li, M.; Xiao, L. Exosome-transmitted circ_MMP2 promotes hepatocellular carcinoma metastasis by upregulating MMP2. *Mol. Oncol.* **2020**, *14*, 1365–1380. [CrossRef]

114. Zhu, C.; Su, Y.; Liu, L.; Wang, S.; Liu, Y.; Wu, J. Circular RNA hsa_circ_0004277 Stimulates Malignant Phenotype of Hepatocellular Carcinoma and Epithelial-Mesenchymal Transition of Peripheral Cells. *Front. Cell Dev. Biol.* **2020**, *8*, 585565. [CrossRef]
115. Chen, C.; Luo, F.; Liu, X.; Lu, L.; Xu, H.; Yang, Q.; Xue, J.; Shi, L.; Li, J.; Zhang, A.; et al. NF-kB-regulated exosomal miR-155 promotes the inflammation associated with arsenite carcinogenesis. *Cancer Lett.* **2017**, *388*, 21–33. [CrossRef]
116. Wu, Q.; Zhou, L.; Lv, D.; Zhu, X.; Tang, H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. *J. Hematol. Oncol.* **2019**, *12*, 53. [CrossRef]
117. Gai, X.; Tang, B.; Liu, F.; Wu, Y.; Wang, F.; Jing, Y.; Huang, F.; Jin, D.; Wang, L.; Zhang, H. mTOR/miR-145-regulated exosomal GOLM1 promotes hepatocellular carcinoma through augmented GSK-3β/MMPs. *J. Genet. Genom.* **2019**, *46*, 235–245. [CrossRef]
118. Leonardi, G.C.; Candido, S.; Cervello, M.; Nicolosi, D.; Raiti, F.; Travali, S.; Spandidos, D.A.; Libra, M. The tumor microenvironment in hepatocellular carcinoma. *Int. J. Oncol.* **2012**, *40*, 1733–1747.
119. Tian, Y.; Li, S.; Song, J.; Ji, T.; Zhu, M.; Anderson, G.J.; Wei, J.; Nie, G. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials* **2014**, *35*, 2383–2390. [CrossRef]
120. Conigliaro, A.; Costa, V.; Lo Dico, A.; Saieva, L.; Buccheri, S.; Dieli, F.; Manno, M.; Raccosta, S.; Mancone, C.; Tripodi, M.; et al. CD90+ liver cancer cells modulate endothelial cell phenotype through the release of exosomes containing H19 lncRNA. *Mol. Cancer* **2015**, *14*, 155. [CrossRef]
121. Huang, A.; Dong, J.; Li, S.; Wang, C.; Ding, H.; Li, H.; Su, X.; Ge, X.; Sun, L.; Bai, C.; et al. Exosomal transfer of vasorin expressed in hepatocellular carcinoma cells promotes migration of human umbilical vein endothelial cells. *Int. J. Biol. Sci.* **2015**, *11*, 961–969. [CrossRef]
122. Moh-Moh-Aung, A.; Fujisawa, M.; Ito, S.; Katayama, H.; Ohara, T.; Ota, Y.; Yoshimura, T.; Matsukawa, A. Decreased miR-200b-3p in cancer cells leads to angiogenesis in HCC by enhancing endothelial ERG expression. *Sci. Rep.* **2020**, *10*, 10418. [CrossRef] [PubMed]
123. Ye, L.; Zhang, Q.; Cheng, Y.; Chen, X.; Wang, G.; Shi, M.; Zhang, T.; Cao, Y.; Pan, H.; Zhang, L.; et al. Tumor-derived exosomal HMGB1 fosters hepatocellular carcinoma immune evasion by promoting TIM-1(+) regulatory B cell expansion. *J. Immunother. Cancer* **2018**, *6*, 145. [CrossRef]
124. Robey, R.W.; Pluchino, K.M.; Hall, M.D.; Fojo, A.T.; Bates, S.E.; Gottesman, M.M. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat. Rev. Cancer* **2018**, *18*, 452–464. [CrossRef] [PubMed]
125. Sun, N.; Lee, Y.-T.; Zhang, R.Y.; Kao, R.; Teng, P.-C.; Yang, Y.; Yang, P.; Wang, J.J.; Smalley, M.; Chen, P.-J.; et al. Purification of HCC-specific extracellular vesicles on nanosubstrates for early HCC detection by digital scoring. *Nat. Commun.* **2020**, *11*, 4489. [CrossRef]
126. Kooijmans, S.A.; Vader, P.; van Dommelen, S.M.; van Solinge, W.W.; Schiffelers, R.M. Exosome mimetics: A novel class of drug delivery systems. *Int. J. Nanomed.* **2012**, *7*, 1525–1541.
127. Khamisipour, G.; Jadidi-Niaragh, F.; Jahromi, A.S.; Zandi, K.; Hojjat-Farsangi, M. Mechanisms of tumor cell resistance to the current targeted-therapy agents. *Tumor Biol.* **2016**, *37*, 10021–10039. [CrossRef]
128. Xue, D.; Han, J.; Liu, Y.; Tuo, H.; Peng, Y. Current perspectives on exosomes in the diagnosis and treatment of hepatocellular carcinoma (review). *Cancer Biol. Ther.* **2021**, *22*, 279–290. [CrossRef]
129. Liu, G.; Ouyang, X.; Sun, Y.; Xiao, Y.; You, B.; Gao, Y.; Yeh, S.; Li, Y.; Chang, C. The miR-92a-2-5p in exosomes from macrophages increases liver cancer cells invasion via altering the AR/PHLPP/p-AKT/β-catenin signaling. *Cell Death Differ.* **2020**, *27*, 3258–3272. [CrossRef]
130. Wang, Y.; Wang, B.; Xiao, S.; Li, Y.; Chen, Q. miR-125a/b inhibits tumor-associated macrophages mediated in cancer stem cells of hepatocellular carcinoma by targeting CD90. *J Cell Biochem* **2019**, *120*, 3046–3055. [CrossRef]
131. Wang, G.; Luo, G.; Zhao, M.; Miao, H. Significance of exosomes in hepatocellular carcinoma. *Front. Oncol.* **2022**, *12*, 1056379. [CrossRef]
132. Tian, X.-P.; Wang, C.-Y.; Jin, X.-H.; Li, M.; Wang, F.-W.; Huang, W.-J.; Yun, J.-P.; Xu, R.-H.; Cai, Q.-Q.; Xie, D. Acidic microenvironment up-regulates exosomal miR-21 and miR-10b in early-stage hepatocellular carcinoma to promote cancer cell proliferation and metastasis. *Theranostics* **2019**, *9*, 1965–1979. [CrossRef] [PubMed]
133. Vaupel, P.; Schmidberger, H.; Mayer, A. The Warburg effect: Essential part of metabolic reprogramming and central contributor to cancer progression. *Int. J. Radiat. Biol.* **2019**, *95*, 912–919. [CrossRef] [PubMed]
134. Qian, D.; Xie, Y.; Huang, M.; Gu, J. Tumor-derived exosomes in hypoxic microenvironment: Release mechanism, biological function and clinical application. *J. Cancer* **2022**, *13*, 1685–1694. [CrossRef] [PubMed]
135. Fang, T.; Lv, H.; Lv, G.; Li, T.; Wang, C.; Han, Q.; Yu, L.; Su, B.; Guo, L.; Huang, S.; et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. *Nat. Commun.* **2018**, *9*, 191. [CrossRef]
136. Yugawa, K.; Yoshizumi, T.; Mano, Y.; Itoh, S.; Harada, N.; Ikegami, T.; Kohashi, K.; Oda, Y.; Mori, M. Cancer-associated fibroblasts promote hepatocellular carcinoma progression through downregulation of exosomal miR-150-3p. *Eur. J. Surg. Oncol.* **2021**, *47*, 384–393. [CrossRef]
137. Li, S.; Qi, Y.; Huang, Y.; Guo, Y.; Huang, T.; Jia, L. Exosome-derived SNHG16 sponging miR-4500 activates HUVEC angiogenesis by targeting GALNT1 via PI3K/Akt/mTOR pathway in hepatocellular carcinoma. *J. Physiol. Biochem.* **2021**, *77*, 667–682. [CrossRef]
138. Dai, W.; Wang, Y.; Yang, T.; Wang, J.; Wu, W.; Gu, J. Downregulation of exosomal CLEC3B in hepatocellular carcinoma promotes metastasis and angiogenesis via AMPK and VEGF signals. *Cell Commun. Signal.* **2019**, *17*, 1–17. [CrossRef]

139. Wang, W.; Wei, C. Advances in the early diagnosis of hepatocellular carcinoma. *Genes Dis.* **2020**, *7*, 308–319. [CrossRef] [PubMed]
140. Manea, I.; Iacob, R.; Iacob, S.; Cerban, R.; Dima, S.; Oniscu, G.; Popescu, I.; Gheorghe, L. Liquid biopsy for early detection of hepatocellular carcinoma. *Front. Med.* **2023**, *10*, 1218705. [CrossRef]
141. Wang, Y.; Zhang, C.; Zhang, P.; Guo, G.; Jiang, T.; Zhao, X.; Jiang, J.; Huang, X.; Tong, H.; Tian, Y. Serum exosomal microRNAs combined with alpha-fetoprotein as diagnostic markers of hepatocellular carcinoma. *Cancer Med.* **2018**, *7*, 1670–1679. [CrossRef]
142. Chen, S.; Zhang, Y.; Wu, X.; Zhang, C.; Li, G. Diagnostic Value of lncRNAs as Biomarker in Hepatocellular Carcinoma: An Updated Meta-Analysis. *Can. J. Gastroenterol. Hepatol.* **2018**, *2018*, 8410195. [CrossRef]
143. Xu, H.; Chen, Y.; Dong, X.; Wang, X. Serum Exosomal Long Noncoding RNAs ENSG00000258332.1 and LINC00635 for the Diagnosis and Prognosis of Hepatocellular Carcinoma. *Cancer Epidemiol. Biomark. Prev.* **2018**, *27*, 710–716. [CrossRef] [PubMed]
144. Fornari, F.; Ferracin, M.; Trerè, D.; Milazzo, M.; Marinelli, S.; Galassi, M.; Venerandi, L.; Pollutri, D.; Patrizi, C.; Borghi, A.; et al. Circulating microRNAs, miR-939, miR-595, miR-519d and miR-494, Identify Cirrhotic Patients with HCC. *PLoS ONE* **2015**, *10*, e0141448. [CrossRef]
145. Yu, D.; Li, Y.; Wang, M.; Gu, J.; Xu, W.; Cai, H.; Fang, X.; Zhang, X. Exosomes as a new frontier of cancer liquid biopsy. *Mol. Cancer* **2022**, *21*, 56. [CrossRef]
146. Weber, J.A.; Baxter, D.H.; Zhang, S.; Huang, D.Y.; Huang, K.H.; Lee, M.J.; Galas, D.J.; Wang, K. The microRNA spectrum in 12 body fluids. *Clin. Chem.* **2010**, *56*, 1733–1741. [CrossRef]
147. Yoshioka, Y.; Konishi, Y.; Kosaka, N.; Katsuda, T.; Kato, T.; Ochiya, T. Comparative marker analysis of extracellular vesicles in different human cancer types. *J. Extracell. Vesicles* **2013**, *2*, 20424. [CrossRef] [PubMed]
148. Wang, Z.; Chen, J.-Q.; Liu, J.-L.; Tian, L. Exosomes in tumor microenvironment: Novel transporters and biomarkers. *J. Transl. Med.* **2016**, *14*, 1–9. [CrossRef]
149. Larssen, P.; Wik, L.; Czarnewski, P.; Eldh, M.; Löf, L.; Ronquist, K.G.; Dubois, L.; Freyhult, E.; Gallant, C.J.; Oelrich, J.; et al. Tracing cellular origin of human exosomes using multiplex proximity extension assays. *Mol. Cell. Proteom.* **2017**, *16*, 502–511. [CrossRef] [PubMed]
150. Goetzl, L.; Darbinian, N.; Goetzl, E.J. Novel window on early human neurodevelopment via fetal exosomes in maternal blood. *Ann. Clin. Transl. Neurol.* **2016**, *3*, 381–385. [CrossRef]
151. Goetzl, L.; Darbinian, N.; Merabova, N. Noninvasive assessment of fetal central nervous system insult: Potential application to prenatal diagnosis. *Prenat. Diagn.* **2019**, *39*, 609–615. [CrossRef]
152. Goetzl, L.; Merabova, N.; Darbinian, N.; Martirosyan, D.; Poletto, E.; Fugarolas, K.; Menkiti, O. Diagnostic Potential of Neural Exosome Cargo as Biomarkers for Acute Brain Injury. *Ann. Clin. Transl. Neurol.* **2018**, *5*, 4–10. [CrossRef] [PubMed]
153. Goetzl, E.J.; Boxer, A.; Schwartz, J.B.; Abner, E.L.; Petersen, R.C.; Miller, B.L.; Kapogiannis, D. Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. *Neurology* **2015**, *85*, 40–47. [CrossRef] [PubMed]
154. Newman, L.A.; Useckaite, Z.; Johnson, J.; Sorich, M.J.; Hopkins, A.M.; Rowland, A. Selective Isolation of Liver-Derived Extracellular Vesicles Redefines Performance of miRNA Biomarkers for Non-Alcoholic Fatty Liver Disease. *Biomedicines* **2022**, *10*, 195. [CrossRef] [PubMed]
155. Zeng, Y.; Hu, S.; Luo, Y.; He, K. Exosome Cargos as Biomarkers for Diagnosis and Prognosis of Hepatocellular Carcinoma. *Pharmaceutics* **2023**, *15*, 2365. [CrossRef] [PubMed]
156. Wang, S.; Xu, M.; Li, X.; Su, X.; Xiao, X.; Keating, A.; Zhao, R.C. Exosomes released by hepatocarcinoma cells endow adipocytes with tumor-promoting properties. *J. Hematol. Oncol.* **2018**, *11*, 82. [CrossRef]
157. Liu, W.-h.; Ren, L.-n.; Wang, X.; Wang, T.; Zhang, N.; Gao, Y.; Luo, H.; Navarro-Alvarez, N.; Tang, L.-J. Combination of exosomes and circulating microRNAs may serve as a promising tumor marker complementary to alpha-fetoprotein for early-stage hepatocellular carcinoma diagnosis in rats. *J. Cancer Res. Clin. Oncol.* **2015**, *141*, 1767–1778. [CrossRef] [PubMed]
158. Li, X.; Li, C.; Zhang, L.; Wu, M.; Cao, K.; Jiang, F.; Chen, D.; Li, N.; Li, W. The significance of exosomes in the development and treatment of hepatocellular carcinoma. *Mol. Cancer* **2020**, *19*, 1. [CrossRef] [PubMed]
159. Chapuy-Regaud, S.; Dubois, M.; Plisson-Chastang, C.; Bonnefois, T.; Lhomme, S.; Bertrand-Michel, J.; You, B.; Simoneau, S.; Gleizes, P.-E.; Flan, B.; et al. Characterization of the lipid envelope of exosome encapsulated HEV particles protected from the immune response. *Biochimie* **2017**, *141*, 70–79. [CrossRef] [PubMed]
160. Haraszti, R.A.; Didiot, M.-C.; Sapp, E.; Leszyk, J.; Shaffer, S.A.; Rockwell, H.E.; Gao, F.; Narain, N.R.; DiFiglia, M.; Kiebish, M.A.; et al. High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. *J. Extracell. Vesicles* **2016**, *5*, 32570. [CrossRef]
161. Llorente, A.; Skotland, T.; Sylvänne, T.; Kauhanen, D.; Róg, T.; Orłowski, A.; Vattulainen, I.; Ekroos, K.; Sandvig, K. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochim. Biophys. Acta* **2013**, *1831*, 1302–1309. [CrossRef]
162. Trajkovic, K.; Hsu, C.; Chiantia, S.; Rajendran, L.; Wenzel, D.; Wieland, F.; Schwille, P.; Brügger, B.; Simons, M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* **2008**, *319*, 1244–1247. [CrossRef] [PubMed]
163. Subra, C.; Laulagnier, K.; Perret, B.; Record, M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie* **2007**, *89*, 205–212. [CrossRef] [PubMed]
164. Sanchez, J.I.; Jiao, J.; Kwan, S.Y.; Veillon, L.; Warmoes, M.O.; Tan, L.; Odewole, M.; Rich, N.E.; Wei, P.; Lorenzi, P.L.; et al. Lipidomic Profiles of Plasma Exosomes Identify Candidate Biomarkers for Early Detection of Hepatocellular Carcinoma in Patients with Cirrhosis. *Cancer Prev. Res.* **2021**, *14*, 955–962. [CrossRef] [PubMed]

165. Van Niel, G.; d'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 213–228. [CrossRef] [PubMed]
166. Xie, J.X.; Fan, X.; Drummond, C.A.; Majumder, R.; Xie, Y.; Chen, T.; Liu, L.; Haller, S.T.; Brewster, P.S.; Dworkin, L.D.; et al. MicroRNA profiling in kidney disease: Plasma versus plasma-derived exosomes. *Gene* **2017**, *627*, 1–8. [CrossRef] [PubMed]
167. Zhang, Q.; Li, H.; Liu, Y.; Li, J.; Wu, C.; Tang, H. Exosomal Non-Coding RNAs: New Insights into the Biology of Hepatocellular Carcinoma. *Curr. Oncol.* **2022**, *29*, 5383–5406. [CrossRef]
168. Cho, H.J.; Eun, J.W.; Baek, G.O.; Seo, C.W.; Ahn, H.R.; Kim, S.S.; Cho, S.W.; Cheong, J.Y. Serum exosomal microRNA, miR-10b-5p, as a potential diagnostic biomarker for early-stage hepatocellular carcinoma. *J. Clin. Med.* **2020**, *9*, 281. [CrossRef] [PubMed]
169. Cho, H.J.; Baek, G.O.; Seo, C.W.; Ahn, H.R.; Sung, S.; Son, J.A.; Kim, S.S.; Cho, S.W.; Jang, J.W.; Nam, S.W.; et al. Exosomal microRNA-4661-5p–based serum panel as a potential diagnostic biomarker for early-stage hepatocellular carcinoma. *Cancer Med.* **2020**, *9*, 5459–5472. [CrossRef] [PubMed]
170. Shi, M.; Jiang, Y.; Yang, L.; Yan, S.; Wang, Y.G.; Lu, X.J. Decreased levels of serum exosomal miR-638 predict poor prognosis in hepatocellular carcinoma. *J. Cell. Biochem.* **2018**, *119*, 4711–4716. [CrossRef]
171. Li, W.; Ding, X.; Wang, S.; Xu, L.; Yin, T.; Han, S.; Geng, J.; Sun, W. Downregulation of serum exosomal miR-320d predicts poor prognosis in hepatocellular carcinoma. *J. Clin. Lab. Anal.* **2020**, *34*, e23239. [CrossRef]
172. Yan, L.; Chen, Y.; Zhou, J.; Zhao, H.; Zhang, H.; Wang, G. Diagnostic value of circulating cell-free DNA levels for hepatocellular carcinoma. *Int. J. Infect. Dis.* **2018**, *67*, 92–97. [CrossRef] [PubMed]
173. Tian, B.W.; Han, C.L.; Dong, Z.R.; Tan, S.Y.; Wang, D.X.; Li, T. Role of Exosomes in Immunotherapy of Hepatocellular Carcinoma. *Cancers* **2022**, *14*, 4036. [CrossRef] [PubMed]
174. Poggio, M.; Hu, T.; Pai, C.-C.; Chu, B.; Belair, C.D.; Chang, A.; Montabana, E.; Lang, U.E.; Fu, Q.; Fong, L.; et al. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell* **2019**, *177*, 414–427.e13. [CrossRef] [PubMed]
175. Kar, R.; Dhar, R.; Mukherjee, S.; Nag, S.; Gorai, S.; Mukerjee, N.; Mukherjee, D.; Vatsa, R.; Jadhav, M.C.; Ghosh, A.; et al. Exosome-Based Smart Drug Delivery Tool for Cancer Theranostics. *ACS Biomater. Sci. Eng.* **2023**, *9*, 577–594. [CrossRef] [PubMed]
176. Kim, M.S.; Haney, M.J.; Zhao, Y.; Mahajan, V.; Deygen, I.; Klyachko, N.L.; Inskoe, E.; Piroyan, A.; Sokolsky, M.; Okolie, O.; et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomed. Nanotechnol. Biol. Med.* **2016**, *12*, 655–664. [CrossRef] [PubMed]
177. Kowal, J.; Tkach, M.; Théry, C. Biogenesis and secretion of exosomes. *Curr. Opin. Cell Biol.* **2014**, *29*, 116–125. [CrossRef] [PubMed]
178. Hadla, M.; Palazzolo, S.; Corona, G.; Caligiuri, I.; Canzonieri, V.; Toffoli, G.; Rizzolio, F. Exosomes increase the therapeutic index of doxorubicin in breast and ovarian cancer mouse models. *Nanomedicine* **2016**, *11*, 2431–2441. [CrossRef]
179. Wei, H.; Chen, J.; Wang, S.; Fu, F.; Zhu, X.; Wu, C.; Liu, Z.; Zhong, G.; Lin, J. A nanodrug consisting of doxorubicin and exosome derived from mesenchymal stem cells for osteosarcoma treatment in vitro. *Int. J. Nanomed.* **2019**, *14*, 8603–8610. [CrossRef]
180. Najafi, S.; Majidpoor, J.; Mortezaee, K. Extracellular vesicle-based drug delivery in cancer immunotherapy. *Drug Deliv. Transl. Res.* **2023**, *13*, 2790–2806. [CrossRef]
181. Morad, G.; Helmink, B.A.; Sharma, P.; Wargo, J.A. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade. *Cell* **2021**, *184*, 5309–5337. [CrossRef]
182. Seo, N.; Akiyoshi, K.; Shiku, H. Exosome-mediated regulation of tumor immunology. *Cancer Sci.* **2018**, *109*, 2998–3004. [CrossRef] [PubMed]
183. Fan, M.; Liu, H.; Yan, H.; Che, R.; Jin, Y.; Yang, X.; Zhou, X.; Yang, H.; Ge, K.; Liang, X.-J.; et al. A CAR T-inspiring platform based on antibody-engineered exosomes from antigen-feeding dendritic cells for precise solid tumor therapy. *Biomaterials* **2022**, *282*, 121424. [CrossRef] [PubMed]
184. Liu, X.; Wei, Q.; Lu, L.; Cui, S.; Ma, K.; Zhang, W.; Ma, F.; Li, H.; Fu, X.; Zhang, C. Immunomodulatory potential of mesenchymal stem cell-derived extracellular vesicles: Targeting immune cells. *Front. Immunol.* **2023**, *14*, 1094685. [CrossRef] [PubMed]
185. Fu, W.; Lei, C.; Liu, S.; Cui, Y.; Wang, C.; Qian, K.; Li, T.; Shen, Y.; Fan, X.; Lin, F.; et al. CAR exosomes derived from effector CAR-T cells have potent antitumour effects and low toxicity. *Nat. Commun.* **2019**, *10*, 4355. [CrossRef] [PubMed]
186. Taghikhani, A.; Farzaneh, F.; Sharifzad, F.; Mardpour, S.; Ebrahimi, M.; Hassan, Z.M. Engineered Tumor-Derived Extracellular Vesicles: Potentials in Cancer Immunotherapy. *Front. Immunol.* **2020**, *11*, 221. [CrossRef] [PubMed]
187. Yan, R.; Chen, H.; Selaru, F.M. Extracellular Vesicles in Hepatocellular Carcinoma: Progress and Challenges in the Translation from the Laboratory to Clinic. *Medicina* **2023**, *59*, 1599. [CrossRef] [PubMed]
188. Jesus, S.; Soares, E.; Cruz, M.T.; Borges, O. Exosomes as adjuvants for the recombinant hepatitis B antigen: First report. *Eur. J. Pharm. Biopharm.* **2018**, *133*, 1–11. [CrossRef] [PubMed]
189. Jang, S.C.; Economides, K.D.; Moniz, R.J.; Sia, C.L.; Lewis, N.; McCoy, C.; Zi, T.; Zhang, K.; Harrison, R.A.; Lim, J.; et al. ExoSTING, an extracellular vesicle loaded with STING agonists, promotes tumor immune surveillance. *Commun. Biol.* **2021**, *4*, 497. [CrossRef] [PubMed]
190. Xu, J.; Ji, L.; Liang, Y.; Wan, Z.; Zheng, W.; Song, X.; Gorshkov, K.; Sun, Q.; Lin, H.; Zheng, X.; et al. CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1. *Signal Transduct. Target. Ther.* **2020**, *5*, 298. [CrossRef] [PubMed]
191. Lu, J.C.; Zhang, P.F.; Huang, X.Y.; Guo, X.J.; Gao, C.; Zeng, H.Y.; Zheng, Y.M.; Wang, S.W.; Cai, J.B.; Sun, Q.M.; et al. Amplification of spatially isolated adenosine pathway by tumor-macrophage interaction induces anti-PD1 resistance in hepatocellular carcinoma. *J. Hematol. Oncol.* **2021**, *14*, 200. [CrossRef]

192. Wang, J.; Yang, Y.; Lu, J.; Wang, X. The role of exosomes in therapeutic resistance of hepatocellular carcinoma. *Hepatoma Res.* **2023**, *9*, 46. [CrossRef]
193. He, C.; Dong, X.; Zhai, B.; Jiang, X.; Dong, D.; Li, B.; Jiang, H.; Xu, S.; Sun, X. MiR-21 mediates sorafenib resistance of hepatocellular carcinoma cells by inhibiting autophagy via the PTEN/Akt pathway. *Oncotarget* **2015**, *6*, 28867–28881. [CrossRef] [PubMed]
194. Lou, G.; Song, X.; Yang, F.; Wu, S.; Wang, J.; Chen, Z.; Liu, Y. Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. *J. Hematol. Oncol.* **2015**, *8*, 122. [CrossRef] [PubMed]
195. Gao, Y.; Yin, Z.; Qi, Y.; Peng, H.; Ma, W.; Wang, R.; Li, W. Golgi phosphoprotein 3 promotes angiogenesis and sorafenib resistance in hepatocellular carcinoma via upregulating exosomal miR-494-3p. *Cancer Cell Int.* **2022**, *22*, 35. [CrossRef] [PubMed]
196. Escudier, B.; Dorval, T.; Chaput, N.; André, F.; Caby, M.-P.; Novault, S.; Flament, C.; Leboulaire, C.; Borg, C.; Amigorena, S.; et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: Results of thefirst phase I clinical trial. *J. Transl. Med.* **2005**, *3*, 10. [CrossRef] [PubMed]
197. Besse, B.; Charrier, M.; Lapierre, V.; Dansin, E.; Lantz, O.; Planchard, D.; Le Chevalier, T.; Livartoski, A.; Barlesi, F.; Laplanche, A.; et al. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. *Oncoimmunology* **2016**, *5*, e1071008. [CrossRef] [PubMed]
198. Gehrmann, U.; Näslund, T.I.; Hiltbrunner, S.; Larssen, P.; Gabrielsson, S. (Eds.) Harnessing the exosome-induced immune response for cancer immunotherapy. In *Seminars in Cancer Biology*; Elsevier: Amsterdam, The Netherlands, 2014.
199. Katzenstein, H.M.; Langham, M.R.; Malogolowkin, M.H.; Krailo, M.D.; Towbin, A.J.; McCarville, M.B.; Finegold, M.J.; Ranganathan, S.; Dunn, S.; McGahren, E.D.; et al. Minimal adjuvant chemotherapy for children with hepatoblastoma resected at diagnosis (AHEP0731): A Children's Oncology Group, multicentre, phase 3 trial. *Lancet Oncol.* **2019**, *20*, 719–727. [CrossRef] [PubMed]
200. Yeung, C.C.; Dondelinger, F.; Schoof, E.M.; Georg, B.; Lu, Y.; Zheng, Z.; Zhang, J.; Hannibal, J.; Fahrenkrug, J.; Kjaer, M. Circadian regulation of protein cargo in extracellular vesicles. *Sci. Adv.* **2022**, *8*, eabc9061. [CrossRef] [PubMed]
201. Partch, C.L.; Green, C.B.; Takahashi, J.S. Molecular architecture of the mammalian circadian clock. *Trends Cell Biol.* **2014**, *24*, 90–99. [CrossRef]
202. Patke, A.; Young, M.W.; Axelrod, S. Molecular mechanisms and physiological importance of circadian rhythms. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 67–84. [CrossRef]
203. Takahashi, J.S. Transcriptional architecture of the mammalian circadian clock. *Nat. Rev. Genet.* **2017**, *18*, 164–179. [CrossRef] [PubMed]
204. Shafi, A.A.; Knudsen, K.E. Cancer and the circadian clock. *Cancer Res.* **2019**, *79*, 3806–3814. [CrossRef] [PubMed]
205. Sancar, A.; Van Gelder, R.N. Clocks, cancer, and chronochemotherapy. *Science* **2021**, *371*, eabb0738. [CrossRef] [PubMed]
206. Buhr, E.D.; Takahashi, J.S. Molecular components of the Mammalian circadian clock. *Handb. Exp. Pharmacol.* **2013**, *217*, 3–27.
207. Panda, S.; Hogenesch, J.B.; Kay, S.A. Circadian rhythms from flies to human. *Nature* **2002**, *417*, 329–335. [CrossRef] [PubMed]
208. Bass, J.; Takahashi, J.S. Circadian integration of metabolism and energetics. *Science* **2010**, *330*, 1349–1354. [CrossRef]
209. Battaglin, F.; Chan, P.; Pan, Y.; Soni, S.; Qu, M.; Spiller, E.R.; Castanon, S.; Torres, E.T.R.; Mumenthaler, S.M.; Kay, S.A.; et al. Clocking cancer: The circadian clock as a target in cancer therapy. *Oncogene* **2021**, *40*, 3187–3200. [CrossRef] [PubMed]
210. Chen, L.; Zhao, J.; Tang, Q.; Li, H.; Zhang, C.; Yu, R.; Zhao, Y.; Huo, Y.; Wu, C. PFKFB3 control of cancer growth by responding to circadian clock outputs. *Sci. Rep.* **2016**, *6*, 24324. [CrossRef]
211. Tang, Q.; Cheng, B.; Xie, M.; Chen, Y.; Zhao, J.; Zhou, X.; Chen, L. Circadian clock gene Bmal1 inhibits tumorigenesis and increases paclitaxel sensitivity in tongue squamous cell carcinoma. *Cancer Res.* **2017**, *77*, 532–544. [CrossRef]
212. Lucassen, E.A.; Coomans, C.P.; van Putten, M.; de Kreij, S.R.; van Genugten, J.H.; Sutorius, R.P.; de Rooij, K.E.; van der Velde, M.; Verhoeve, S.L.; Smit, J.W.; et al. Environmental 24-hr cycles are essential for health. *Curr. Biol.* **2016**, *26*, 1843–1853. [CrossRef]
213. Pan, A.; Schernhammer, E.S.; Sun, Q.; Hu, F.B. Rotating night shift work and risk of type 2 diabetes: Two prospective cohort studies in women. *PLoS Med.* **2011**, *8*, e1001141. [CrossRef] [PubMed]
214. Logan, R.W.; McClung, C.A. Rhythms of life: Circadian disruption and brain disorders across the lifespan. *Nat. Rev. Neurosci.* **2019**, *20*, 49–65. [CrossRef] [PubMed]
215. Dooner, M.S.; Stewart, C.; Deng, Y.; Papa, E.; Pereira, M.; Del Tatto, M.; Johnson, S.; Wen, S.; Amaral, A.; Aliotta, J.; et al. Daily rhythms influence the ability of lung-derived extracellular vesicles to modulate bone marrow cell phenotype. *PLoS ONE* **2018**, *13*, e0207444. [CrossRef] [PubMed]
216. Khalyfa, A.; Gaddameedhi, S.; Crooks, E.; Zhang, C.; Li, Y.; Qiao, Z.; Trzepizur, W.; Kay, S.A.; Andrade, J.; Satterfield, B.C.; et al. Circulating Exosomal miRNAs Signal Circadian Misalignment to Peripheral Metabolic Tissues. *Int. J. Mol. Sci.* **2020**, *21*, 6396. [CrossRef] [PubMed]
217. Danielson, K.M.; Estanislau, J.; Tigges, J.; Toxavidis, V.; Camacho, V.; Felton, E.J.; Khoory, J.; Kreimer, S.; Ivanov, A.R.; Mantel, Y.; et al. Diurnal Variations of Circulating Extracellular Vesicles Measured by Nano Flow Cytometry. *PLoS ONE* **2016**, *11*, e0144678. [CrossRef] [PubMed]
218. Kumar, A.; Sundaram, K.; Mu, J.; Dryden, G.W.; Sriwastva, M.K.; Lei, C.; Zhang, L.; Qiu, X.; Xu, F.; Yan, J.; et al. High-fat diet-induced upregulation of exosomal phosphatidylcholine contributes to insulin resistance. *Nat. Commun.* **2021**, *12*, 213. [CrossRef]
219. Noh, J. The Effect of Circadian and Sleep Disruptions on Obesity Risk. *J. Obes. Metab. Syndr.* **2018**, *27*, 78–83. [CrossRef] [PubMed]

220. Chaput, J.-P.; McHill, A.W.; Cox, R.C.; Broussard, J.L.; Dutil, C.; da Costa, B.G.G.; Sampasa-Kanyinga, H.; Wright, K.P. The role of insufficient sleep and circadian misalignment in obesity. *Nat. Rev. Endocrinol.* **2023**, *19*, 82–97. [CrossRef] [PubMed]
221. Koritzinsky, E.H.; Street, J.M.; Chari, R.R.; Glispie, D.M.; Bellomo, T.R.; Aponte, A.M.; Star, R.A.; Yuen, P.S.T. Circadian variation in the release of small extracellular vesicles can be normalized by vesicle number or TSG101. *Am. J. Physiol. Physiol.* **2019**, *317*, F1098–F1110. [CrossRef]
222. Eckel-Mahan, K.L.; Patel, V.R.; De Mateo, S.; Orozco-Solis, R.; Ceglia, N.J.; Sahar, S.; Dilag-Penilla, S.A.; Dyar, K.A.; Baldi, P.; Sassone-Corsi, P. Reprogramming of the circadian clock by nutritional challenge. *Cell* **2013**, *155*, 1464–1478. [CrossRef]
223. Vollmers, C.; Gill, S.; DiTacchio, L.; Pulivarthy, S.R.; Le, H.D.; Panda, S. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21453–21458. [CrossRef] [PubMed]
224. Watanabe, M.; Tuccinardi, D.; Ernesti, I.; Basciani, S.; Mariani, S.; Genco, A.; Manfrini, S.; Lubrano, C.; Gnessi, L. Scientific evidence underlying contraindications to the ketogenic diet: An update. *Obes. Rev.* **2020**, *21*, e13053. [CrossRef] [PubMed]
225. Tognini, P.; Murakami, M.; Liu, Y.; Eckel-Mahan, K.L.; Newman, J.C.; Verdin, E.; Baldi, P.; Sassone-Corsi, P. Distinct circadian signatures in liver and gut clocks revealed by ketogenic diet. *Cell Metab.* **2017**, *26*, 523–538.e5. [CrossRef] [PubMed]
226. Ribas-Latre, A.; Fekry, B.; Kwok, C.; Baumgartner, C.; Shivshankar, S.; Sun, K.; Chen, Z.; Eckel-Mahan, K. Rosiglitazone reverses high fat diet-induced changes in BMAL1 function in muscle, fat, and liver tissue in mice. *Int. J. Obes.* **2019**, *43*, 567–580. [CrossRef] [PubMed]
227. Lamia, K.A.; Storch, K.-F.; Weitz, C.J. Physiological significance of a peripheral tissue circadian clock. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 15172–15177. [CrossRef] [PubMed]
228. Zhang, E.E.; Liu, Y.; Dentin, R.; Pongsawakul, P.Y.; Liu, A.C.; Hirota, T.; Nusinow, D.A.; Sun, X.; Landais, S.; Kodama, Y.; et al. Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. *Nat. Med.* **2010**, *16*, 1152–1156. [CrossRef]
229. Erren, T.C.; Falaturi, P.; Morfeld, P.; Knauth, P.; Reiter, R.J.; Piekarski, C. Shift work and cancer: The evidence and the challenge. *Dtsch. Arztebl. Int.* **2010**, *107*, 657–662. [PubMed]
230. Erren, T.C.; Morfeld, P.; Groß, J.V.; Wild, U.; Lewis, P. "Night shift work" is probably carcinogenic: What about disturbed chronobiology in all walks of life? *J. Occup. Med. Toxicol.* **2019**, *14*, 29. [CrossRef] [PubMed]
231. Kinouchi, K.; Sassone-Corsi, P. Metabolic rivalry: Circadian homeostasis and tumorigenesis. *Nat. Rev. Cancer* **2020**, *20*, 645–661. [CrossRef]
232. Koritala, B.S.; Porter, K.I.; Arshad, O.A.; Gajula, R.P.; Mitchell, H.D.; Arman, T.; Manjanatha, M.G.; Teeguarden, J.; Van Dongen, H.P.A.; McDermott, J.E.; et al. Night shift schedule causes circadian dysregulation of DNA repair genes and elevated DNA damage in humans. *J. Pineal Res.* **2021**, *70*, e12726. [CrossRef]
233. Chen, W.-D.; Wen, M.-S.; Shie, S.-S.; Lo, Y.-L.; Wo, H.-T.; Wang, C.-C.; Hsieh, I.C.; Lee, T.H.; Wang, C.Y. The circadian rhythm controls telomeres and telomerase activity. *Biochem. Biophys. Res. Commun.* **2014**, *451*, 408–414. [CrossRef] [PubMed]
234. Ruan, W.; Yuan, X.; Eltzschig, H.K. Circadian rhythm as a therapeutic target. *Nat. Rev. Drug Discov.* **2021**, *20*, 287–307. [CrossRef] [PubMed]
235. Huang, C.; Zhang, C.; Cao, Y.; Li, J.; Bi, F. Major roles of the circadian clock in cancer. *Cancer Biol. Med.* **2023**, *20*, 1–24. [CrossRef] [PubMed]
236. Kettner, N.M.; Voicu, H.; Finegold, M.J.; Coarfa, C.; Sreekumar, A.; Putluri, N.; Katchy, C.A.; Lee, C.; Moore, D.D.; Fu, L. Circadian homeostasis of liver metabolism suppresses hepatocarcinogenesis. *Cancer Cell* **2016**, *30*, 909–924. [CrossRef] [PubMed]
237. Ye, Y.; Xiang, Y.; Ozguc, F.M.; Kim, Y.; Liu, C.-J.; Park, P.K.; Hu, Q.; Diao, L.; Lou, Y.; Lin, C.; et al. The genomic landscape and pharmacogenomic interactions of clock genes in cancer chronotherapy. *Cell Syst.* **2018**, *6*, 314–328.e2. [CrossRef] [PubMed]
238. Dong, Z.; Zhang, G.; Qu, M.; Gimple, R.C.; Wu, Q.; Qiu, Z.; Prager, B.C.; Wang, X.; Kim, L.J.; Morton, A.R.; et al. Targeting Glioblastoma Stem Cells through Disruption of the Circadian Clock. *Cancer Discov.* **2019**, *9*, 1556–1573. [CrossRef] [PubMed]
239. Chen, P.; Hsu, W.-H.; Chang, A.; Tan, Z.; Lan, Z.; Zhou, A.; Spring, D.J.; Lang, F.F.; Wang, Y.A.; DePinho, R.A. Circadian regulator CLOCK recruits immune-suppressive microglia into the GBM tumor microenvironment. *Cancer Discov.* **2020**, *10*, 371–381. [CrossRef] [PubMed]
240. Papagiannakopoulos, T.; Bauer, M.R.; Davidson, S.M.; Heimann, M.; Subbaraj, L.; Bhutkar, A.; Bartlebaugh, J.; Heiden, M.G.V.; Jacks, T. Circadian rhythm disruption promotes lung tumorigenesis. *Cell Metab.* **2016**, *24*, 324–331. [CrossRef]
241. Qu, M.; Zhang, G.; Qu, H.; Vu, A.; Wu, R.; Tsukamoto, H.; Jia, Z.; Huang, W.; Lenz, H.-J.; Rich, J.N.; et al. Circadian regulator BMAL1::CLOCK promotes cell proliferation in hepatocellular carcinoma by controlling apoptosis and cell cycle. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2214829120. [CrossRef]
242. Fekry, B.; Ribas-Latre, A.; Drunen, R.V.; Santos, R.B.; Shivshankar, S.; Dai, Y.; Zhao, Z.; Yoo, S.; Chen, Z.; Sun, K.; et al. Hepatic circadian and differentiation factors control liver susceptibility for fatty liver disease and tumorigenesis. *Faseb J.* **2022**, *36*, e22482. [CrossRef]
243. Crespo, M.; Leiva, M.; Sabio, G. Circadian Clock and Liver Cancer. *Cancers* **2021**, *13*, 3631. [CrossRef] [PubMed]
244. Fekry, B.; Ribas-Latre, A.; Baumgartner, C.; Deans, J.R.; Kwok, C.; Patel, P.; Fu, L.; Berdeaux, R.; Sun, K.; Kolonin, M.G.; et al. Incompatibility of the circadian protein BMAL1 and HNF4α in hepatocellular carcinoma. *Nat. Commun.* **2018**, *9*, 4349. [CrossRef] [PubMed]
245. Zisapel, N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *Br. J. Pharmacol.* **2018**, *175*, 3190–3199. [CrossRef] [PubMed]

246. Talib, W.H.; Alsayed, A.R.; Abuawad, A.; Daoud, S.; Mahmod, A.I. Melatonin in Cancer Treatment: Current Knowledge and Future Opportunities. *Molecules* **2021**, *26*, 2506. [CrossRef]
247. Cheng, L.; Liu, J.; Liu, Q.; Liu, Y.; Fan, L.; Wang, F.; Yu, H.; Li, Y.; Bu, L.; Li, X.; et al. Exosomes from melatonin treated hepatocellularcarcinoma cells alter the immunosupression status through STAT3 pathway in macrophages. *Int. J. Biol. Sci.* **2017**, *13*, 723. [CrossRef] [PubMed]
248. Zhang, X.; Pant, S.M.; Ritch, C.C.; Tang, H.-Y.; Shao, H.; Dweep, H.; Gong, Y.-Y.; Brooks, R.; Brafford, P.; Wolpaw, A.J.; et al. Cell state dependent effects of Bmal1 on melanoma immunity and tumorigenicity. *Nat. Commun.* **2024**, *15*, 633. [CrossRef] [PubMed]
249. Schibler, U.; Sassone-Corsi, P. A web of circadian pacemakers. *Cell* **2002**, *111*, 919–922. [CrossRef] [PubMed]
250. Ray, S.; Valekunja, U.K.; Stangherlin, A.; Howell, S.A.; Snijders, A.P.; Damodaran, G.; Reddy, A.B. Circadian rhythms in the absence of the clock gene Bmal1. *Science* **2020**, *367*, 800–806. [CrossRef]
251. Khalyfa, A.; Poroyko, V.A.; Qiao, Z.; Gileles-Hillel, A.; Khalyfa, A.A.; Akbarpour, M.; Farré, R.; Gozal, D. Exosomes and metabolic function in mice exposed to alternating dark-light cycles mimicking night shift work schedules. *Front. Physiol.* **2017**, *8*, 311175. [CrossRef]
252. Chang, H.C.; Guarente, L. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. *Cell* **2013**, *153*, 1448–1460. [CrossRef]
253. Asher, G.; Gatfield, D.; Stratmann, M.; Reinke, H.; Dibner, C.; Kreppel, F.; Mostoslavsky, R.; Alt, F.W.; Schibler, U. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* **2008**, *134*, 317–328. [CrossRef] [PubMed]
254. Forterre, A.; Jalabert, A.; Chikh, K.; Pesenti, S.; Euthine, V.; Granjon, A.; Errazuriz, E.; Lefai, E.; Vidal, H.; Rome, S. Myotube-derived exosomal miRNAs downregulate Sirtuin1 in myoblasts during muscle cell differentiation. *Cell Cycle* **2014**, *13*, 78–89. [CrossRef]
255. Shi, X.-F.; Wang, H.; Kong, F.-X.; Xu, Q.-Q.; Xiao, F.-J.; Yang, Y.-F.; Ge, R.-L.; Wang, L.-S. Exosomal miR-486 regulates hypoxia-induced erythroid differentiation of erythroleukemia cells through targeting Sirt1. *Exp. Cell Res.* **2017**, *351*, 74–81. [CrossRef]
256. Ding, L.; Li, Z.-l.; Zhou, Y.; Liu, N.-c.; Liu, S.-s.; Zhang, X.-j.; Liu, C.-C.; Zhang, D.-J.; Wang, G.-H.; Ma, R.-X. Loss of Sirt1 promotes exosome secretion from podocytes by inhibiting lysosomal acidification in diabetic nephropathy. *Mol. Cell. Endocrinol.* **2023**, *568–569*, 111913. [CrossRef] [PubMed]
257. Wang, Z.; Guo, W.; Yi, F.; Zhou, T.; Li, X.; Feng, Y.; Guo, Q.; Xu, H.; Song, X.; Cao, L. The Regulatory Effect of SIRT1 on Extracellular Microenvironment Remodeling. *Int. J. Biol. Sci.* **2021**, *17*, 89–96. [CrossRef] [PubMed]
258. McAndrews, K.M.; LeBleu, V.S.; Kalluri, R. SIRT1 Regulates Lysosome Function and Exosome Secretion. *Dev. Cell* **2019**, *49*, 302–303. [CrossRef] [PubMed]
259. Roy, S.; Das, A.; Vernekar, M.; Mandal, S.; Chatterjee, N. Understanding the Correlation between Metabolic Regulator SIRT1 and Exosomes with CA-125 in Ovarian Cancer: A Clinicopathological Study. *BioMed Res. Int.* **2022**, *2022*, 5346091. [CrossRef]
260. Liu, L.; Jin, X.; Hu, C.-F.; Li, R.; Zhou, Z.; Shen, C.-X. Exosomes derived from mesenchymal stem cells rescue myocardial ischaemia/reperfusion injury by inducing cardiomyocyte autophagy via AMPK and Akt pathways. *Cell. Physiol. Biochem.* **2017**, *43*, 52–68. [CrossRef]
261. Liu, F.; Bu, Z.; Zhao, F.; Xiao, D. Increased T-helper 17 cell differentiation mediated by exosome-mediated micro RNA-451 redistribution in gastric cancer infiltrated T cells. *Cancer Sci.* **2018**, *109*, 65–73. [CrossRef]
262. Arslan, F.; Lai, R.C.; Smeets, M.B.; Akeroyd, L.; Choo, A.; Aguor, E.N.; Timmers, L.; Van Rijen, H.V.; Doevendans, P.A.; Pasterkamp, G.; et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res.* **2013**, *10*, 301–312. [CrossRef]
263. Mukherji, A.; Bailey, S.M.; Staels, B.; Baumert, T.F. The circadian clock and liver function in health and disease. *J. Hepatol.* **2019**, *71*, 200–211. [CrossRef] [PubMed]
264. Li, X.; Guan, J.; Jiang, Z.; Cheng, S.; Hou, W.; Yao, J.; Wang, Z. Microglial Exosome miR-7239-3p Promotes Glioma Progression by Regulating Circadian Genes. *Neurosci. Bull.* **2021**, *37*, 497–510. [CrossRef] [PubMed]
265. Qu, M.; Qu, H.; Jia, Z.; Kay, S.A. HNF4A defines tissue-specific circadian rhythms by beaconing BMAL1::CLOCK chromatin binding and shaping the rhythmic chromatin landscape. *Nat. Commun.* **2021**, *12*, 6350. [CrossRef] [PubMed]
266. Deans, J.R.; Deol, P.; Titova, N.; Radi, S.H.; Vuong, L.M.; Evans, J.R.; Pan, S.; Fahrmann, J.; Yang, J.; Hammock, B.D.; et al. HNF4α isoforms regulate the circadian balance between carbohydrate and lipid metabolism in the liver. *Front. Endocrinol.* **2023**, *14*, 1266527. [CrossRef] [PubMed]
267. Zhang, H.; Zhang, X.; Wang, S.; Zheng, L.; Guo, H.; Ren, Y.; Qiao, B.; Wu, J.; Zhao, D.; Xu, L.; et al. Adipocyte-derived exosomal miR-22-3p modulated by circadian rhythm disruption regulates insulin sensitivity in skeletal muscle cells. *J. Biol. Chem.* **2023**, *299*, 105476. [CrossRef] [PubMed]
268. Pusic, A.D.; Kraig, R.P. Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. *Glia* **2014**, *62*, 284–299. [CrossRef] [PubMed]
269. Tao, S.C.; Guo, S.C. Extracellular Vesicles: Potential Participants in Circadian Rhythm Synchronization. *Int. J. Biol. Sci.* **2018**, *14*, 1610–1620. [CrossRef] [PubMed]
270. Shende, V.R.; Goldrick, M.M.; Ramani, S.; Earnest, D.J. Expression and rhythmic modulation of circulating microRNAs targeting the clock gene Bmal1 in mice. *PLoS ONE* **2011**, *6*, e22586. [CrossRef] [PubMed]

271. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* **2003**, *13*, 2498–2504. [CrossRef]
272. Warde-Farley, D.; Donaldson, S.L.; Comes, O.; Zuberi, K.; Badrawi, R.; Chao, P.; Franz, M.; Grouios, C.; Kazi, F.; Lopes, C.T.; et al. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* **2010**, *38* (Suppl. S2), W214–W220. [CrossRef]
273. Liu, W.; Yu, M.; Xie, D.; Wang, L.; Ye, C.; Zhu, Q.; Liu, F.; Yang, L. Melatonin-stimulated MSC-derived exosomes improve diabetic wound healing through regulating macrophage M1 and M2 polarization by targeting the PTEN/AKT pathway. *Stem Cell Res. Ther.* **2020**, *11*, 1–15. [CrossRef] [PubMed]
274. Yoon, Y.M.; Lee, J.H.; Song, K.H.; Noh, H.; Lee, S.H. Melatonin-stimulated exosomes enhance the regenerative potential of chronic kidney disease-derived mesenchymal stem/stromal cells via cellular prion proteins. *J. Pineal Res.* **2020**, *68*, e12632. [CrossRef] [PubMed]
275. Zhou, Z.; Wang, R.; Wang, J.; Hao, Y.; Xie, Q.; Wang, L.; Wang, X. Melatonin pretreatment on exosomes: Heterogeneity, therapeutic effects, and usage. *Front. Immunol.* **2022**, *13*, 933736. [CrossRef] [PubMed]
276. Wei, J.X.; Lv, L.H.; Wan, Y.L.; Cao, Y.; Li, G.L.; Lin, H.M.; Zhou, R.; Shang, C.Z.; Cao, J.; He, H.; et al. Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells. *Hepatology* **2015**, *61*, 1284–1294. [CrossRef] [PubMed]
277. Qiu, Y.; Li, P.; Zhang, Z.; Wu, M. Insights into Exosomal Non-Coding RNAs Sorting Mechanism and Clinical Application. *Front. Oncol.* **2021**, *11*, 664904.
278. Lee, Y.J.; Shin, K.J.; Chae, Y.C. Regulation of cargo selection in exosome biogenesis and its biomedical applications in cancer. *Exp. Mol. Med.* **2024**, *56*, 877–889. [CrossRef] [PubMed]
279. Garcia-Martin, R.; Brandao, B.B.; Thomou, T.; Altindis, E.; Kahn, C.R. Tissue differences in the exosomal/small extracellular vesicle proteome and their potential as indicators of altered tissue metabolism. *Cell Rep.* **2022**, *38*, 110277. [CrossRef] [PubMed]
280. Liu, W.-Z.; Ma, Z.-J.; Kang, X.-W. Current status and outlook of advances in exosome isolation. *Anal. Bioanal. Chem.* **2022**, *414*, 7123–7141. [CrossRef]
281. Gao, J.; Li, A.; Hu, J.; Feng, L.; Liu, L.; Shen, Z. Recent developments in isolating methods for exosomes. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1100892. [CrossRef]
282. Jin, X.; Zhang, J.; Zhang, Y.; He, J.; Wang, M.; Hei, Y.; Guo, S.; Xu, X.; Liu, Y. Different origin-derived exosomes and their clinical advantages in cancer therapy. *Front. Immunol.* **2024**, *15*, 1401852. [CrossRef]
283. Li, X.; Corbett, A.L.; Taatizadeh, E.; Tasnim, N.; Little, J.P.; Garnis, C.; Daugaard, M.; Guns, E.; Hoorfar, M.; Li, I.T.S. Challenges and opportunities in exosome research-Perspectives from biology, engineering, and cancer therapy. *APL Bioeng.* **2019**, *3*, 011503. [CrossRef] [PubMed]
284. Mathivanan, S.; Ji, H.; Simpson, R.J. Exosomes: Extracellular organelles important in intercellular communication. *J. Proteom.* **2010**, *73*, 1907–1920. [CrossRef] [PubMed]
285. Innominato, P.F.; Roche, V.P.; Palesh, O.G.; Ulusakarya, A.; Spiegel, D.; Lévi, F.A. The circadian timing system in clinical oncology. *Ann. Med.* **2014**, *46*, 191–207. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.