

# Exosomal delivery of TRAIL and miR-335 for the treatment of hepatocellular carcinoma (Review)

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**Abstract.** Liver cancer is the sixth most prevalent type of cancer worldwide and accounts for the third most frequent cause of cancer-associated mortality. Conventional anticancer drugs display limited efficacy owing to their short half-life, poor solubility and inefficient drug delivery. Despite advancements being made in drug discovery and development for the treatment of hepatocellular carcinoma (HCC), drug inefficacy and drug continue to pose significant obstacles to effective treatment. Therefore, it is imperative that novel treatment strategies be developed with the aim of developing anticancer treatments without any side-effects and with long-term durability. Extracellular vesicles, such as exosomes, intercellular communication agents which have the ability to carry heterogeneous molecules with high penetrability, low immunogenicity and longer durability, may provide a versatile natural delivery system. The present review article illustrates the innovative treatment strategy using exosomes as a delivery agent for two distinct anticancer candidates, i.e., tumor necrosis factor-related apoptosis-inducing ligand and microRNA-335. The aim of the present review was to present a unique strategy for the development of an exceptional anticancer treatment therapy exploiting exosomes as a delivery vehicle which may be used for HCC.

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## 1. Introduction

Liver cancer is the sixth most prevalent form of cancer worldwide and accounts for the third most frequent cause of cancer-associated mortality (1,2) (Fig. 1). Hepatocellular carcinoma (HCC) is the most predominant form of malignant liver cancer, which is responsible for ~90% of all non-metastatic liver cancers and has been reported to be associated with cirrhosis and hepatitis B or C virus (HBV/HCV) infection (3,4). Other risk factors include obesity, iron overload, alcohol consumption, diabetes, fatty liver disease and smoking (5).

However, according to statistics, only 25% of patients with HCC are diagnosed in the initial stages at the onset of the disease (6). A probable reason for this may be the absence of initial symptoms and frequent overlap with other diseases, thus making it difficult to distinguish HCC from other clinical conditions. The survival rates of cancer patients may be markedly enhanced by timely and precise diagnosis at the initial stages (7). As with the late detection of advanced-stage HCC, the diagnosis of cancer at a late stage suggests that the patient has reached a stage that is non-curative. Henceforth, the chances of survival are greatly minimized, and such patients are placed under palliative care due to the high rate of metastasis and relapse, as occurs in patients with late-stage HCC (8,9).

At present, the most common strategies for the treatment of HCC include ablation, surgical resection, liver transplantation, chemotherapy, transarterial chemoembolization (TACE), radiotherapy and combination therapy depending on the disease staging and patient's profile (Fig. 1) (10). However, these conventional therapies have multiple limitations that

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compromise the quality of life of patients receiving these therapies. For example, undesired effects of radiotherapy and the development of resistance to chemotherapy due to long-term treatment and transplantation lead to long-term immunosuppressive therapy (11). Although substantial advancements have been made over the past decade in the management and treatment of HCC, including liver resection or transplantation and ablation, only ~15% of patients with early-stage HCC without cirrhosis are eligible for surgical removal. TACE is another available treatment option for patients with intermediate-stage HCC that results in a 23% increase in the 2-year survival rate when compared to traditional treatment therapies (12).

Most HCC cases are diagnosed predominantly in the late stages of the disease, which renders both surgical (resection and transplantation) and locoregional treatment (chemoembolization) inadequate for the overall survival of patients. As a result, there is an urgent need for the development of an effective therapy for patients with advanced-stage HCC.

In the SHARP randomized controlled trial, sorafenib as a monotherapy was shown to be efficacious for advanced HCC. With a high safety profile, the sorafenib-treated group exhibited an overall survival rate of 10.7 months compared to 7.9 months for the placebo group (13) (Fig. 2). Sorafenib is currently the only approved prescribed option for the treatment of patients with advanced-stage HCC. Patients with HCC who have not responded to earlier treatments are recommended to use sorafenib, which received authorization by the US Food and Drug Administration (FDA) in 2007 (14). The anticancer effect of sorafenib is based on its ability to obstruct cell proliferation and angiogenesis, which inhibits tumor growth (15). However, sorafenib treatment is beneficial to only a limited number of patients and is often accompanied by drug resistance within 6 months of commencing the treatment. Moreover, the use of sorafenib is also associated with side-effects, such as nausea, alopecia and hypertension (14).

It took >10 years following the approval of sorafenib before a second first-line targeted drug for HCC was developed. As per the outcome from REFLECT trial (16), a randomized phase III non-inferiority trial reported by Kudo *et al* (16), led to the approval of lenvatinib to be used as the first-line treatment for advanced HCC.

In terms of overall survival, this randomized phase III trial in 2018 demonstrated that lenvatinib was not inferior to sorafenib showing overall survival of 13.6 months compared to 12.6 months for the sorafenib-treated group (16). A recent milestone in the development of HCC first-line drug development was achieved in 2020 when the FDA approved bevacizumab plus atezolizumab, an antibody combination strategy, as a first-line treatment for patients with unresectable HCC on the basis of safety and efficacy determined in the IMbrave150 trial (17). In this phase III study, 501 patients with HCC who had not previously received systemic treatment were compared to the effectiveness of bevacizumab coupled with atezolizumab against sorafenib. By significantly improving the overall survival by 12.6% at 12 months, the combination treatment significantly outperformed sorafenib monotherapy (17). Although all three drugs approved by the FDA for first-line therapy had led to an improved response and survival rate, they are all associated with multiple adverse effects (Fig. 2).

Despite advancements being made in several first- and second-line drugs for the treatment of HCC, the use of these drugs still presents the issue of a compromised lifestyle with the provision of less benefit overall. The quality of life of patients receiving therapy with these drugs does not appear to be improving, and there is also the issue of the high costs of these drugs. Moreover, these targeted therapies are associated with the drawback of an inadequate objective response rate (ORR) and adaptive/acquired resistance (18). Furthermore, the long-term usage of such chemotherapeutic drugs may pose the issue of toxicity, as well as drug inefficacy. Therefore, it is imperative that a novel treatment strategy be developed with the aim of developing targeted therapy that can be applied to patients with HCC at any stage of treatment without posing any side-effects with longer durability.

The present review article illustrates the innovative exosome-based therapy as a delivery agent of two potential anticancer candidates, i.e., tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and microRNA (miRNA/miR)-335. The summary and discussion of scientific investigations highlights the immense potential of harnessing the ability of exosomes for developing an effective anticancer drug therapy for patients with liver cancer and their role in addressing the issue of drug resistance development conferred by existing chemotherapeutics.

## 2. MicroRNA-335: A novel candidate for anticancer treatment

miRNAs are non-coding RNAs whose function is to perform post-transcriptional gene regulation. They play a key role in tumor development by modulating the expression of various oncogenes and tumor suppressor genes (19). miRNAs are poorly regulated in multiple types of cancers and, as per their function, they function either as oncogenes or tumor suppressors. Oncomirs, such as miR-21 stimulate tumor growth by impeding tumor suppressor genes (20). On the contrary, tumor suppressor miRNAs, such as miR-145 restrict the progression of tumors by blocking oncogenes (21). Furthermore, miRNAs are involved in several biological processes, including cell proliferation, apoptosis, metastasis and angiogenesis (22,23), which are the key features in cancer progression.

miRNAs as a novel class of regulatory factors are crucial to the biological and pathological processes in several cases of human solid tumors (24). According to numerous recent studies, as a tumor suppressor gene, miR-335-5p has been linked to the emergence and growth of several tumors, including colorectal cancer (25), non-small cell lung cancer (26) and HCC (27). Furthermore, hepatic stellate cell-derived miR-335-5p exosomes may be used as prospective miRNA biomarkers in HBV-related HCC (28) and thus have potential therapeutic significance in HCC (29).

Previous research has demonstrated that miR-335 is a suppressor of tumor formation, invasion and metastasis, and is responsible for the regulation of apoptosis and thus has prognostic value in HCC (30). miR-335-3p, sometimes referred to as miR-335\*, is generated concurrently with miR-335. Notably, these miRNAs have also been shown to be responsible for inducing the activation of the p53 tumor suppressor pathway to prevent cellular proliferation and neoplastic cell

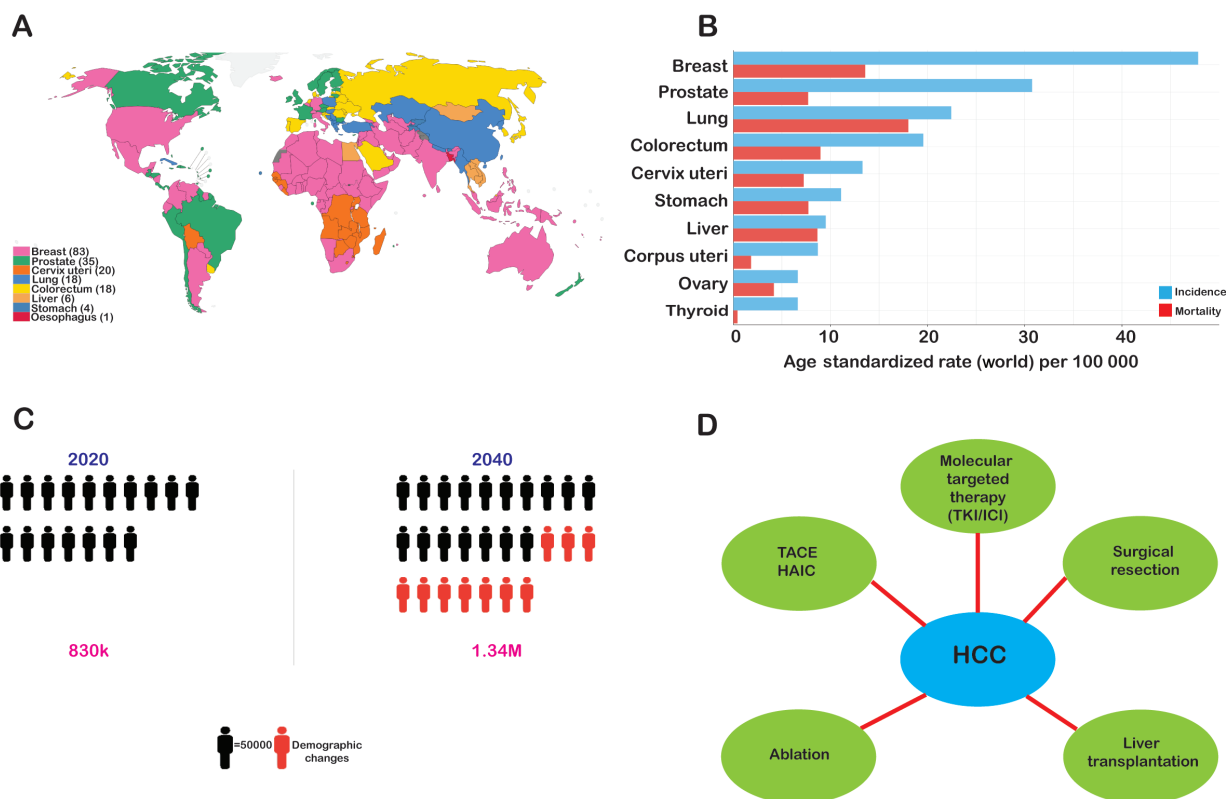


Figure 1. Worldwide Epidemiology of Liver Cancer in 2022 (International Agency for Research on Cancer) Cancer Today; <https://gco.iarc.fr/today/home>; accessed October 21, 2022). (A) The estimated incidences of different types of cancer worldwide in 2022. (B) Histogram representation of the estimated number of incident cases and deaths worldwide. (C) Number of deaths from liver cancer from 2020 to 2040 as estimated by the World Health Organization. (D) Current treatment approaches for HCC. HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; HAIC, hepatic arterial infusion chemotherapy; TKI, tyrosine kinase inhibitor; ICI, immune checkpoint inhibitor.

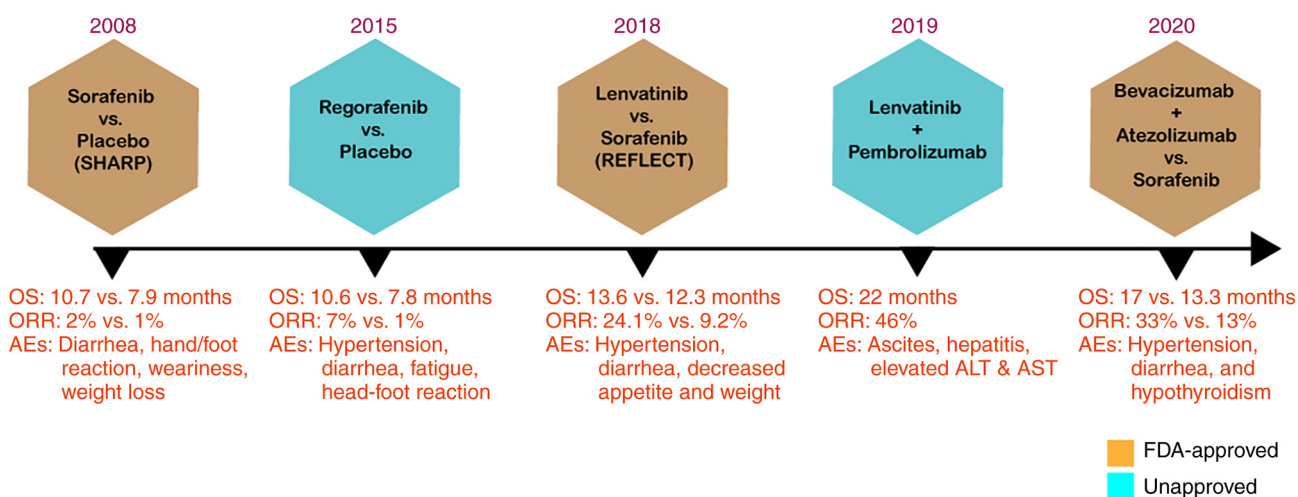


Figure 2. Timeline of FDA-approved drugs for HCC. The SHARP trial (13) demonstrated the effectiveness of sorafenib against HCC. Compared to the placebo group, the sorafenib-treated group exhibited a markedly longer OS (mOS 10.7 vs. 7.9 months; HR, 0.69; 95% CI, 0.55-0.87;  $P < 0.001$ ). In the REFLECT trial (16), lenvatinib displayed non-inferiority in OS corresponding to sorafenib monotherapy (mOS, 13.6 vs. 12.3 months; HR, 0.92; 95% CI, 0.79-1.06). In the IMbravel150 trial (17), the efficacy of bevacizumab combined with atezolizumab was compared with that of sorafenib. The combination treatment resulted in a markedly improved outcome than sorafenib monotherapy, exhibiting prolonged OS and PFS (mOS 19.2 vs. 13.4 months; HR, 0.66; 95% CI, 0.52-0.85;  $P = 0.0009$ ). FDA, Food and Drug Administration; HCC, hepatocellular carcinoma; OS, overall survival; HR, hazard ratio; Mos, median OS; CI, confidence interval; ORR, objective response rate; PFS, progress free survival; AEs, adverse effects.

transformation (31). Emerging evidence has depicted the role of miR-335 in the majority of oncogenic signaling pathways responsible for cell growth and survival. It has been discovered that the downregulation of miR-335 in lung cancer enhances

cell proliferation by activating the AKT/mTOR signaling pathway, which is one of the most commonly dysregulated signaling pathways in human malignancies (32). This characteristic of miR-335 could be exploited in therapeutic

applications for restricting HCC progression and controlling metastasis.

miR-335-5p reportedly targets downstream genes to modulate the biological activity of cancer cells. For example, miR-335-5p overexpression has been shown to suppress the proliferation, migration and invasion of non-small cell lung cancer by targeting CPNE1 (33). In the case of HCC, miR-335-5p has been reported to significantly attenuate the development of this type of cancer. For example, a previous study demonstrated how the circ\_0009910/miR-335-5p/Rho-associated coiled-coil-containing protein kinase 1 (ROCK1) axis is crucial to the onset and development of HCC (34). In that study, miR-335-5p inhibited HCC cell proliferation, migration and invasion by targeting ROCK1. By enhancing the inhibitory effects of miR-335-5p on the expression of ROCK1 in HCC, circ\_0009910 knockdown exhibited anticancer properties. In addition to that, it was found that the invasiveness of cancer cells was positively associated with ROCK1 (34). In a similar context, Liu *et al* (35) demonstrated that miR-335 was involved in suppressing HCC cell proliferation, migration and invasion by downregulating ROCK1 expression. Their research investigating molecular mechanisms revealed that ROCK1, which is associated with cell movement and invasion in various cancer types, was a target gene for miR-335 for modulating the proliferation and metastasis of HCC cells (35). Reportedly, ROCK1 functions as an oncogene in HCC and promotes the development of HCC (36).

The significance of miR-335 was further validated by another study which demonstrated that miR-335 restoration inhibited hepatic stellate cell (HSC) migration (37). The findings of that study concluded that miR-335 considerably decreased during HSC activation. Restoring miR-335 expression markedly decreased collagen type I and  $\alpha$ -smooth muscle actin levels and prevented cell migration, at least in part through the downregulation of tenascin-C, an extracellular matrix glycoprotein involved in cell migration. The overexpression of miR-335 in HSC may thus provide a novel strategy for the treatment of hepatic fibrosis (37). Another recent study by Yang *et al* (38) revealed that a newly characterized circular RNA, circ\_0005075, promoted HCC progression by suppressing the function of miR-335. Circ\_0005075 was discovered to be upregulated in HCC tissues where it was found that the downregulation of circ\_0005075 inhibited HCC progression (38). As per their study mitogen-activated protein kinase 1 (MAPK1) was shown to be regulated by miR-335, divulging it as one of the downstream regulatory targets of circ\_0005075. It was also shown that the upregulation of circ\_0005075 may be responsible for the elevated level of MAPK1 (38). As a crucial member of the MAPK family, the main function of MAPK1 involves cell proliferation, gene expression, differentiation, mitosis, cell survival and apoptosis (39). Likewise, their role is also imperative in tumorigenesis. With the overexpression of MAPK1 in multiple types of cancer, including breast cancer (40), they could be the likely candidates to be used for the prognosis of patients with HCC (38). To sum up, Yang *et al* (38) demonstrated that miR-335 may target and inhibit MAPK1 (38). Moreover, Ji *et al* (41) identified octamer-binding transcription factor 4 (OCT4) as another target gene of miR-335-5p. miR-335-5p was demonstrated to prevent OCT4 gene expression to restrict the

downstream activation of the Akt signaling pathway, which was demonstrated to be a canonical regulator in the development of liver cancer (41).

Notably, a number of scientific investigations have also discovered a pertinent link between miR-335 expression and the survival of cancer patients (26,42). Furthermore, several findings have indicated that miR-335 influences the chemotherapeutic response in patients receiving standard treatment (43,44). For example, the study by Cui *et al* (45) demonstrated that serum miR-335 levels can be utilized as a marker to identify the status of disease progression, apart from clinical outcome in patients receiving TACE therapy. TACE therapy is the standard of care treatment for patients with large or multinodular HCC whose treatment response varies and still lacks any prognostic marker. However, the study by Cui *et al* (45) indicated that low levels of miR-335 in patient serum were associated with a low survival rate with a poor treatment response. Chen and Xia (46) demonstrated the role of miR-335 as a biomarker for HCC treatment and demonstrated its function responsible for regulating sensitivity to sorafenib in HCC. They depicted the fact that miR-335 regulates sorafenib sensitivity in HCC cells by inhibiting the AKT pathway via targeting C-MET, which is a tyrosine kinase protein involved in the development of cancer (46).

Dohi *et al* (47) first reported that miR-335, which is located within the intron of its protein-coding host gene, MEST, was downregulated due to aberrant promoter hypermethylation. Primary HCC tissues exhibited considerably higher levels of miR-335/MEST methylation and miR-335 expression was much lower in tumors compared to the non-tumor tissue counterparts (47). Their finding suggested that aberrant DNA methylation in primary HCC was the cause of the decreased miR-335 expression. Furthermore, their findings suggested that a decreased expression of miR-335 may be linked to distant metastases in HCC (47). Similar findings were also provided on the expression of miR-335 in tumor tissues, which was reported to be much lower than in non-tumor tissues compared to HCC patient samples (48). The recent study by Nie *et al* (49) demonstrated ROCK1 as a target gene of miR-335-5p, where circ\_0064288 enhanced ROCK1 expression by competitively binding with miR-335-5p. They suggested that circ\_0064288, which is highly expressed in HCC, functions as an oncogene by inhibiting miR-335-5p expression and promoting ROCK1 expression, which is responsible for regulating cell motility. A list of various studies investigating the clinical significance of miR-335 in HCC and their molecular mechanisms is presented in Table I. Thus, potential diagnostic and treatment options for HCC may be provided via the modulation of miR-335.

### 3. Significance of TRAIL in HCC treatment

TRAIL is a pro-apoptotic ligand that has received increasing attention owing to its property of inducing apoptosis in multiple types of cancer cells without affecting normal cells (50). This unique feature of TRAIL has allowed several researchers to investigate the development of TRAIL-receptor agonists as a form of anticancer therapy (51,52). Additionally, TRAIL-based therapeutics are independent of p53 in tumor cells, unlike other chemotherapeutic drugs, which renders them unique in terms of the cell death pathway (53,54).

Table I. Clinical function and mechanisms of action of miRNA-335 in HCC.

| Clinical significance   | Mechanisms   | Recipient cells   | (Refs.) |
|---|--|---|---------|
| Suppresses HCC cell proliferation, migration and invasion   | By enhancing the inhibitory effects of miR-335-5p on the expression of ROCK1 in HCC            | HepG2, Hep3B, HCCLM3, MHCC97 (human HCC cell lines)                   | (34)    |
| Restricts the proliferation, migration and invasion of HCC cells  | Via downregulating the Rho-associated coiled-coil-containing protein kinase 1                  | HuH7 cells and HepG2  | (35)    |
| circ_0005075 promotes HCC proliferation, migration, invasion, anti-apoptosis, and chemotherapeutic resistance         | Via repressing the function of miR-335   | HepG2 and SMMC-7721 cells   | (38)    |
| Restricts the proliferation of Huh-7 liver cancer cells   | Via targeting the OCT4/Akt pathway   | Huh7 human liver cancer cells   | (41)    |
| miR-335 contributes to the sensitizing effects of anticancer drugs  | SIAH2 is the target of miR-335 by enhancing the expression of HDAC3                            | SNU387R, Malme3MR, SNU387Rtaxol, Malme3MR-Taxol, SNU387-R Vinblastine | (43)    |
| Prognostic marker   | Via aberrant promoter hypermethylation   | Patients with HCC   | (45)    |
| Modulates sorafenib resistance  | Via suppressing the c-Met-Akt pathway through lncRNA NEAT1                                     | HepG2/Bel7404   | (46)    |
| Diminishes the expression of miR-335, which may be associated with distant metastasis in HCC                          | DNA hypermethylation of CpG islands within promoter regions of protein-coding host gene, MEST  | Huh1, Huh7, HLE, HLF and HepG2  | (48)    |
| miR-335-5p overexpression partly counteracts the effect of circ_0064288 responsible for HCC cell growth and migration | Circ_0064288 facilitates HCC cell growth and migration by regulating the miR-335-5p/ROCK1 axis | Huh7, Hep3B, HCCLM3, and MHCC97-L                                     | (49)    |
| Obstructs the proliferation and invasion, and increases the apoptosis of HCC cells                                    | Shuttle between hepatoma cells and HSCs, downregulate mRNA targets for miR-335                 | MHCC97L, MHCC97H, Huh7 and HepG2 cells                                | (137)   |

HCC, hepatocellular carcinoma; OCT4, octamer-binding transcription factor 4; SIAH2, Siah E3 ubiquitin protein ligase 2; HDAC3, histone deacetylase 3; ROCK1, Rho-associated coiled-coil-containing protein kinase 1.

TRAIL is a transmembrane protein reported to be found on natural killer (NK) cells and cytotoxic T-cell surfaces with a predominant expression in tissues, including the prostate, lungs and spleen (55). TRAIL can be secreted in the soluble form, which is non-toxic to normal cells, while healthy adult plasma contains a trace quantity of endogenous TRAIL (100 pg/ml) (56). Multiple types of cancer cells overexpress the death receptors (DRs), DR4 and DR5 (57). Upon secretion from NK cells, TRAIL binds to DR4 and DR5 (58) and upon binding, it leads to the recruitment of caspase-8 to the Fas-associated death domain adaptor protein. Following activation, it culminates in apoptotic signaling via caspase-3 activation, ultimately leading to cell death (Fig. 3) (59).

The ability of TRAIL to induce tumor-specific cell death renders it a promising candidate for antitumor therapy. In numerous *in vitro* studies, TRAIL protein in its soluble

recombinant form has been widely explored as an anticancer agent (57,60-62). A number of previous publications with the aim of developing antitumor therapy have centered their findings on the *in vitro* and *in vivo* tumoricidal activity of TRAIL protein; they demonstrated a peculiar feature of apoptosis induction by recombinant, soluble TRAIL protein in a broad range of cancer cell lines, although they demonstrated no activity against normal cells (63-65). The safe use of TRAIL as a ligand for therapeutic purposes, displaying no noticeable cytotoxicity to normal tissues, has also been validated in mouse models (57) and in humans (66).

The study by Grisendi *et al* (67) on adipose-derived (AD)-mesenchymal stem cells (MSCs) producing TRAIL revealed that when AD-MSCs loaded with TRAIL were injected into mice, they localized to the tumor site and induced apoptosis without causing any significant toxicity

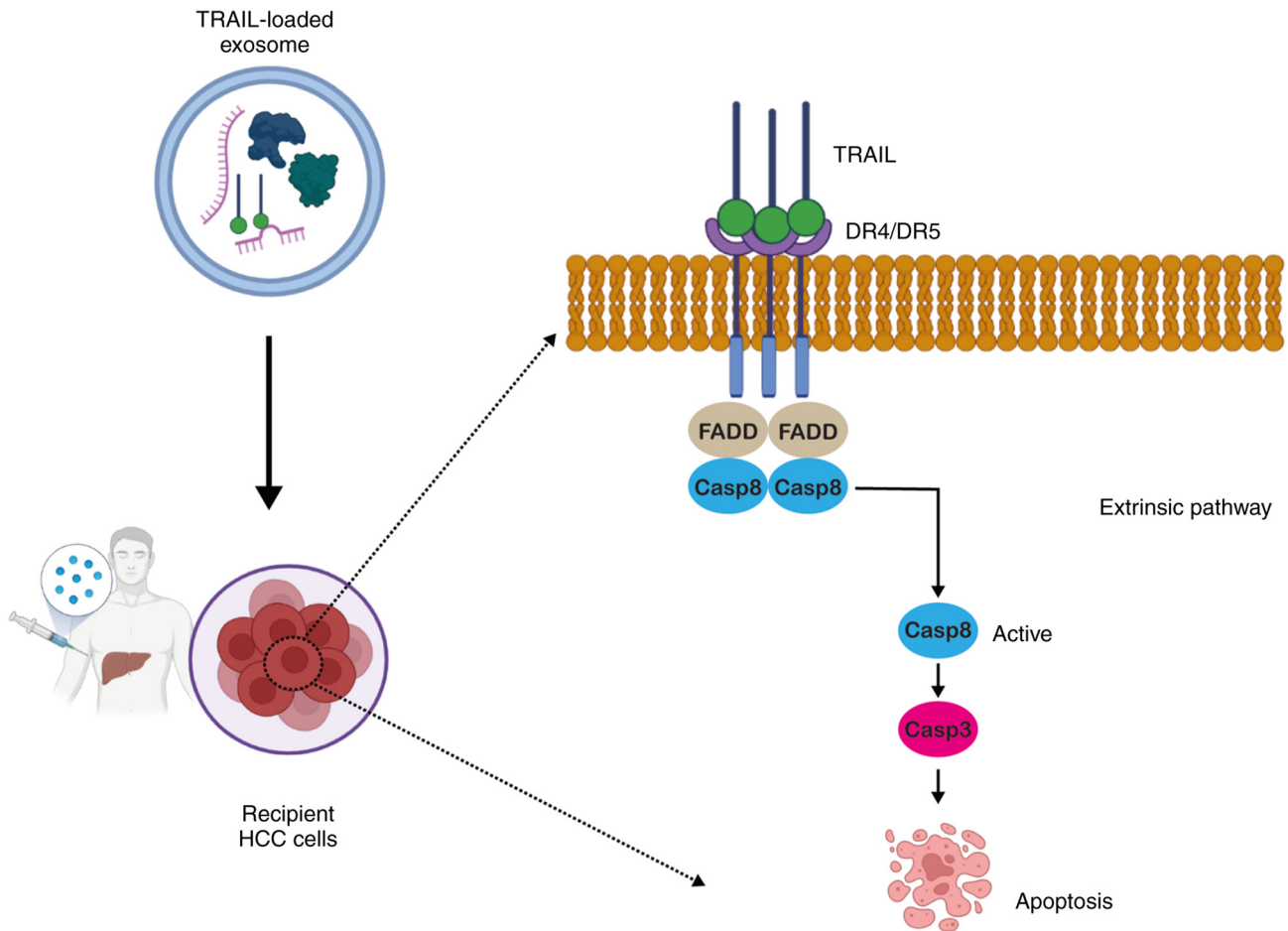


Figure 3. Schematic representation of the exosomal delivery of TRAIL and TRAIL-mediated cell death in recipient HCC cells. TRAIL binds to its cognate DR4 or DR5 on target cancer cells. Upon binding, it leads to the recruitment of caspase-8 to the FADD adaptor protein. Following activation, it culminates in apoptosis signaling via caspase-3-activation, ultimately leading to cell death. The figure was created using BioRender.com. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DR, death receptor; FADD, Fas-associated death domain; HCC, hepatocellular carcinoma; Casp, caspase.

to normal tissues. Those authors also proposed that using stably genetically modified AD-MSCs to deliver TRAIL alone or in conjunction with sensitizing drugs may provide new treatment options for malignancies that remain incurable (67). Furthermore, El-Shemi *et al* (61) revealed TRAIL and inhibitor of growth 4 (ING4) as potent apoptosis-inducing genes in an orthotopic mouse model of human HCC bearing utilizing oncolytic adenoviruses as a gene delivery agent. Their study found that the combination of these drugs significantly reduced tumor-driven angiogenesis and neovascularization, and also triggered apoptosis and immune responses, without exhibiting any overlapping toxicities (61). Another study by Liu *et al* (65) revealed that TRAIL plasmid DNA delivered via HCC-targeted lipid/calcium/phosphate/protamine nanoparticles in conjunction with traditional sorafenib therapy decreased HCC development, as well as liver fibrosis in a mouse model of HCC. Overall, these findings provide a promising treatment strategy for cancer based on TRAIL that may be applied in clinical settings.

Another study recently demonstrated the underlying mechanisms of action of TRAIL, which involved causing substantial cytotoxicity to tumor cells only, but seldom affecting non-transformed cells (68). That research revealed an interaction between TRAIL and immediate early response

gene (IER3), which is expressed in a number of human tissues, and appears to be downregulated in cancer cells, and its overexpression can stimulate the apoptosis of cancer cells and enhance their sensitivity to chemotherapeutic drugs (68). It was demonstrated that these two proteins may be responsible for directing HCC cells to undergo apoptosis and interrupt with their capacity to proliferate and migrate. These findings demonstrate that TRAIL can partly influence the pathogenesis of HCC by interacting with IER3 to reduce Wnt/-catenin signaling (68). A number of TRAIL-based therapeutics for the treatment of HCC are currently undergoing or have undergone clinical trials, as demonstrated in Table II.

**Challenges with TRAIL therapy.** Unfortunately, despite being such a prominent feature of being specific to tumor cells, TRAIL-based therapy still has a long way to go for successful clinical translation. TRAIL-based therapy, including recombinant or agonistic monoclonal antibodies against DR4/DR5 (69,70), has exhibited limited efficacy in a number of clinical trials of different stages due to the short plasma half-life of recombinant TRAIL, limited bioavailability and undesirable systemic toxicity (69). Moreover, TRAIL-based therapy is associated with several challenges, including the development of TRAIL-mediated cell death resistance that

Table II. List of clinical trials conducted on TRAIL-based therapy against various types of cancer.

| Cancer type   | Mechanism   | Settings  | Clinical trial/status  |
|---|---|---|--|
| Advanced-stage HCC  | Monoclonal antibody targeting TRAIL-R1 (mapatumumab)                                      | Combination therapy (sorafenib)                         | Phase II completed (143) (NCT01258608)   |
| Advanced non-small cell lung cancer   | Apoptosis-inducing recombinant TRAIL via DR4 and DR5 activation (mapatumumab.)            | Combination therapy (Paclitaxel and Carboplatin)        | Phase III completed in 2018 (144) (NCT00583830)  |
| Relapsed and refractory multiple myeloma  | Recombinant TRAIL triggering apoptosis via the activation of DR4 and DR5 (CPT)            | Combination therapy (thalidomide)                       | Phase III completed in 2014 (ChiCTRONC-1200206)  |
| Advanced-stage HCC  | TRAIL receptor agonists against TRAIL-R1(DR4)   | Combination of mapatumumab with sorafenib               | Phase II (completed in 2013) (NCT01258608)   |
| Metastatic triple-negative breast cancer  | Monoclonal antibody targeting TRAIL-R2 (tigatuzumab)                                      | Combination therapy (abraxane)                          | Phase II (completed in 2017) (NCT01307891)   |
| Non-small cell lung cancer  | Targeted stem cells expressing TRAIL (MSC TRAIL)  | Combination therapy (pemetrexed/cisplatin chemotherapy) | Phase III clinical trial estimated to be completed in September, 2025 (NCT03298763)          |
| B-cell non-Hodgkin's lymphoma   | Recombinant TRAIL triggering apoptosis via activation of DR4 and DR5 (dulamermin)         | Combination therapy (rituximab)                         | Phase II (completed in 2010) (145) (NCT00118209)   |
| Advanced solid tumors   | TRAIL receptor agonists against DR5 (DS-8273a)  | Monotherapy   | Phase I (completed in 2017) (146) (NCT02076451)  |
| Colorectal cancer<br>non-small cell lung cancer, triple-negative breast cancer, renal cell carcinoma, gastric cancer, pancreatic cancer | Equal mixture of two humanized non-competing DR5-specific monoclonal antibodies (GEN1029) | Monotherapy   | Phase III clinical trial estimated to be completed in March, 2022 (NCT03576131) (terminated) |

HCC, hepatocellular carcinoma; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DR, death receptor.

leads to TRAIL-induced apoptosis being ineffective in HCC. The root cause of the development of therapeutic resistance may be intrinsic resistance in some highly malignant tumors and acquired resistance post-frequent exposure to TRAIL (71). Other factors may include the activation of anti-apoptotic molecules and multiple receptors of various signaling pathways (71-73).

Developing agonist monoclonal antibodies (mAbs) targeting DR4 or DR5 receptors has been the most prevailing approach due to their long serum half-lives *in vivo*. A number of clinical trials, have been conducted on several agonists, such as mapatumumab (NCT01258608), lexatumumab (NCT00428272) and tigatuzumab (NCT01307891); however, none of them displayed any antitumor response rates in cancer patients (69). Similarly, in preclinical tumor xenograft mouse models, mapatumumab and lexatumumab have failed to eradicate tumors (74,75). The dimeric structure of the antibody is most likely to blame for the failure of all agonist mAb clinical trials. Since the binding of ligand on DR4 and DR5 induces

receptor trimerization following the activation of the extrinsic pathway, maintaining its trimeric structure will be required in the future to enable its functional mechanism of apoptosis induction.

The strategy employing recombinant human native TRAIL appears to be more feasible as it allows the preservation of the original trimeric structure with full functional efficacy. Dulamermin, which is Amgen's version of TRAIL, was not found to be effective against cancer in human clinical trials, even though it was effective in preclinical tumor xenograft models (76). The probable reason for the inefficacy of dulamermin may be related to its poor pharmacokinetic profile due to its very short half-life in mammals. Given its low molecular weight and its non-covalently linked trimeric structure instability, which may cause rapid renal elimination. All these facts illustrate the requirement of a novel approach to tackle this challenge.

To obtain such biologically active TRAIL, many expression systems, such as His, Flag tag or the incorporation of trimerization domains, such as leucine zipper or isoleucine

zipper and the stabilization of trimers with cations, as zinc were identified. It has been validated that to assemble and maintain a functionally folded ligand trimer of TRAIL, zinc chelation is critical (77,78). However, it appears to have its own set of drawbacks. For example, both leucine zipper-fused TRAIL and an N-terminus His-tagged TRAIL stabilized by insertion mutation are likely to be immunogenic in humans (57). In addition, particularly the His-tagged version, has been linked to hepatotoxicity not observed with native TRAIL (79,80). Other strategies for prolonging the half-life of TRAIL, which include albumin-conjugated TRAIL nanoparticles or liposome conjugated TRAIL, present the issue of production limitation (81,82).

The recent study by Naval *et al* (83) demonstrated the significance of the oligomerization of TRAIL receptors in TRAIL-induced apoptosis. They stated that TRAIL, as a transmembrane protein, exhibited more potent pro-apoptotic properties than its soluble form (83). In immune system cells, TRAIL is expressed as a type II membrane protein in the plasma membrane (84,85) or is enclosed within microvesicles (86,87). DR5 is solely triggered by the membrane-bound form of TRAIL, whereas DR4 can be activated by both the soluble and membrane-bound forms of the ligand (88). Given the property of TRAIL of being naturally secreted as a membrane protein in exosomes, a number of nanocarriers, such as liposomes, whose lipid composition replicates natural exosomes with surface-bound TRAIL, have been investigated in several pre-clinical studies on its anticancer properties. Various *in vitro* and *in vivo* studies have demonstrated that this membrane-bound form of TRAIL has greater antitumor activity than the soluble form against hematological and solid tumors (89-91). In comparison to soluble TRAIL, this liposomal formulation with TRAIL attached to the liposome surface produces improved DR5 clustering and increased DISC recruitment, resulting in a greater apoptotic signal (90). As TRAIL produces high-order TRAIL oligomers on the lipid nanoparticle surface, improved DR5 clustering and higher DISC recruitment is accomplished (92). Furthermore, TRAIL-encapsulated liposomes and nanoparticles face hurdles with agent release from carriers (93,94). In summary, the therapeutic benefits of TRAIL therapy have been limited, possibly due to the resistance displayed by HCC cells and poor pharmacokinetics. Thus, an effective delivery agent is required for the delivery of TRAIL, which can increase its circulation time in the human body with optimal encapsulation.

#### 4. Exosomes: A novel approach for drug delivery

Conventional anticancer drugs display limited efficacy owing to their short half-life, poor solubility and inefficacious delivery, resulting in the development of drug resistance and substantial systemic toxicity (95). Apart from this, poor drug delivery is also a major contributor to treatment failure. After entering the blood circulation by injection, the drug faces a variety of hurdles before reaching and acting on the target site (96). To improve the efficacy of HCC chemotherapeutics, a drug delivery system with active targeting and local, controlled, and continuous drug release is urgently required.

Extracellular vesicles (EV)-based therapeutics are a promising drug delivery system, since they can penetrate tissues and even cross the blood-brain barrier as natural nanoscale

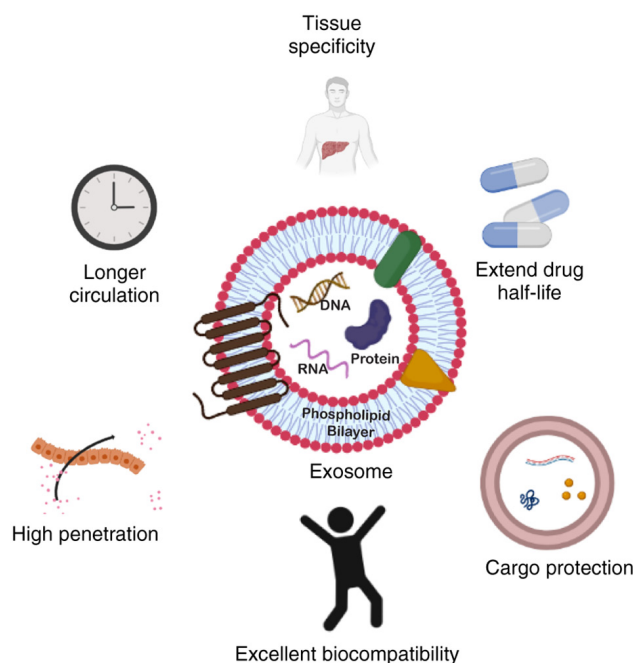


Figure 4. Structure of exosomes and benefits of using exosomes as a drug delivery system compared to other existing agents. The figure was created using BioRender.com.

agents (97,98). Exosomes in particular, offer numerous benefits as drug delivery vehicles, which include a small size, low cytotoxicity, long half-life in the circulation, and the ability to load various cargoes with high a biocompatibility (Fig. 4) (99,100). Conventional drug delivery strategies frequently fall short of the intended results for several reasons, including the rapid *in vivo* degradation of miRNAs, the loss of native structure in proteins and the potential for severe toxicity in normal cells. However, these issues may be resolved by using exosomes as carriers and thus, by deploying exosomes to deliver drugs to tumor sites, an effective and promising approach may be made available for targeted cancer therapy.

Exosomes are endosomal-derived EVs with a size of 30-200 nm that have been reported to be released by a multitude of cell types. They have the characteristic cargo-loading capacity of carrying heterogenous biomolecules, such as DNA, RNA, proteins and lipids, and transferring them to recipient cells, thus acting as intercellular messengers. Exosomes are formed by the intraluminal budding of multivesicular bodies (MVBs) of an intact cell and are released into the extracellular environment when these MVBs fuse with the plasma membrane of the recipient cell (101).

Exosomes have the potential to mirror the intricacy of the parental cell with the innate capacity to regulate multitude of roles in crucial biological activities (102). Consequently, this characteristic led to exosome-based applications in cancer treatment and diagnoses being more feasible. Exosomes contain molecules with a wide range of functions, but lack the complexity of cells and organs; as a result, exosomes are regarded as excellent tools for use in the treatment of a variety of disorders, including cancer. Exosomes also have a number of advantages in terms of biocompatibility, stability, cellular uptake mechanism, biodistribution, pharmacokinetics and immunogenicity, rendering them promising anticancer



candidates. These characteristics can raise the therapeutic index of exosome-based cancer treatments by preferentially targeting tumor cells, while reducing undesirable side-effects. Below is a brief illustration of key facts of the therapeutic potential of exosomes over present drug delivery platforms.

*Source and safety.* MSCs have been reported to be the most favorable selection for the commercial production of exosomes due to the ease of availability and are reported to produce an excessive number of exosomes (EVs) with consistent sustainability and reproducibility compared to other cell lines (103). Most importantly, applying MSC-exosomes as an agent to deliver drugs has been reported to have no safety concerns, including the chances of inducing tumorigenicity (104,105). MSC-derived EVs exhibit significant flexibility for *in vitro* and *in vivo* modifications (106), as well as a high stability in human plasma and at storage at 20°C (107,108).

Furthermore, MSC-derived EVs have been demonstrated to be well-tolerated in a variety of animal models, aside from possessing therapeutic benefits as proven in the treatment of myocardial infarction, chronic kidney disease, wound healing and liver injury in mouse models (109,110). Previous studies have employed EVs as an efficient systemic natural gene carrier for transporting anticancer miRNAs and proteins (111,112). A number of phase I clinical trials have validated the safety of EV administration; no reports of grade II toxicity were reported with the determination of the maximal tolerated dose (113-115).

*Drug delivery and cellular uptake.* As aforementioned, exosomes, being a natural cellular messenger, provide the benefit of a heterogeneous cargo-loading capacity and specificity. Exosomes are known to possess homing properties that can deliver cargo even to distant targets and in between cells with suitable biocompatibility, and to regulate the functions of targeted cells transiently (116). Reportedly, the interaction of exosomes with target cells involves multiple mechanisms, as exosomes can directly bind to membrane receptors of the recipient cell for content internalization, or they can transport bioactive cargo by fusing with the plasma membrane of the target cell.

Presently, a number of drugs face the issue of not being able to cross the blood-brain barrier, limiting the efficacy of several therapies, including cancer therapies. However, exosomes can cross the blood-brain barrier to increase intracranial drug concentration (117). For example, exosomes have been shown to carry medicines or siRNA to the brains of mice with Alzheimer's disease (111,118). Unlike the traditional approach of drug administration, delivery using exosomes does not have the drawback of drug toxicity, intracranial infection and imprecise absorption (118).

*Cargo protection and improved durability.* The ideal delivery agent does not only perform the site-specific transportation of enclosed therapeutics, but should also be capable of protecting the enclosed material and avoiding premature degradation by the body's immune system. Exosomes have a lipid bilayer structure that not only aids in transport efficiency and supports the load of hydrophobic or hydrophilic drugs, but also protects the encapsulated material (119). Furthermore, being able to

have a reduced clearance rate can sustain the drugs in the body's circulation. Exosomes, being natural products of the body, do not invoke any immune response and possess a longer circulation half-life, which can prevent therapeutic cargo from degrading too rapidly (118).

*Stability.* Exosomes are well-known for their stability, as they preserve the identity of their parental cells, while maintaining their long-term innate integrity (107). Multiple freeze-thaw cycles have been shown to have no effect on their size, indicating that freezing has no effect on the quality of exosomes stored (107). Kalra *et al* (108) demonstrated the stability of colon cancer-derived exosomes and noted that the majority of samples retained their integrity even without protease inhibitors for 3 months, and that the highest stability was found at 80°C. This property of being stable for a long period of time in storage at 80°C suggests another advantage of exosomes over existing anticancer drugs (120).

Furthermore, a previous study found that therapeutic exosomes maintained antitumor activity even after being frozen for at least 5 months (121). Exosomes can shield therapeutic nucleic acids and proteins from RNases and proteinase degradation as they contain fragile bioactive molecules within a lipid bilayer membrane (122). In addition, under both physiological and pathological conditions, exosomes appear to display an enhanced stability in the blood, that facilitates long-distance travel within the body. Thus, exosome stability covers not just the human body, but also storage in the field. In addition to this, they have also been shown to have improved stability in the blood, allowing them to cover long distances throughout the body under both normal and pathological conditions (123).

*Therapeutic significance and drug resistance.* It has been demonstrated that drugs encapsulated with exosomes lead to an enhanced chemotherapeutic efficacy (124). Exosomes appear to have a much higher potential (>10-fold) in targeting cancer cells compared to liposomes of similar size (125). Furthermore, currently, the major barrier to an effective therapy or complete cure is multi-drug resistance (MDR). This resistance is commonly shown by all cancer patients undergoing long-term chemotherapy. The exosome is one such natural nanocarrier which has proven to be efficient in overcoming the issue of MDR in tumors. Kim *et al* (126) confirmed this by integrating paclitaxel (PTX) into exosomes released from macrophages for the treatment of MDR cancer. They determined that exosomes augmented cytotoxicity in drug-resistant cells by >50-fold when compared to exosome-free drugs (126). Furthermore, when doxorubicin (DOX)-loaded exosomes were administered intranasally to animals with pulmonary metastasis, confocal fluorescence microscopy revealed an almost perfect co-localization with cancer cells. These findings suggest that PTX-loaded exosomes inhibit MDR tumors and pulmonary metastasis growth more effectively (126).

## 5. Challenges, synergism, and strategies against liver cancer

Several drug delivery strategies have been developed over the years for the treatment of cancer; however, only a few of

these have obtained clinical approval. The likelihood of an efficacious drug delivery to cancer tissues following *in vivo* administration is <0.7% (127). The expected accomplishments in the case of HCC drug development may not be as satisfactory as in the case of other types of cancer. Furthermore, based upon the outcome of recent clinical trials, a single drug therapy appears to be inadequate in the case of treatment for advanced-stage HCC (128). Thus, combination therapy is a major area of research for the treatment of advanced-stage HCC.

Surprisingly, formulations containing recombinant TRAIL have encountered considerable difficulties in being evaluated for human application due to its undesirable systemic toxicity, the short plasma half-life of recombinant TRAIL, or the activation of anti-apoptotic proteins (62,69,94,129). However, the exosome-based encapsulation of TRAIL protein can convey better pharmacokinetic characteristics, greater bioavailability and the ability to cross target tissues to the enclosed protein/drug (130). For example, in a previous study, TRAIL was transduced into leukemia K562 cells with human membrane TRAIL and produced TRAIL secreted exosomes, which were shown to trigger the apoptosis of melanoma and lymphoma cell lines *in vitro* (131). These findings reveal that cells that have been genetically engineered to express TRAIL can secrete exosomes that contain the pro-apoptotic ligand in an active form in their membranes. Although therapeutic success varied in the different tumor models studied, TRAIL exosomes exhibited potent killing activity *in vitro* and *in vivo*, in both local and systemic therapy modalities (131).

Another study by Yuan *et al* (132) demonstrated that TRAIL-loaded exosomes were more efficient in inducing cell death than recombinant soluble TRAIL. It was shown that the fluidic nature of the lipid bilayer membrane in exosomes harboring TRAIL may allow higher order TRAIL oligomerization and, as a result, the stronger clustering of its receptors, which is a crucial signal for effective extrinsic death pathway activation (132). It was demonstrated that the limited bioavailability of TRAIL, the low activity and cell resistance to TRAIL ligand can be overcome by TRAIL-expressing EVs derived from MSCs, thereby improving the clinical efficacy of TRAIL. This EV-loaded TRAIL effectively induced apoptosis in a variety of cancer cell lines, including lung (A549, NCI-H460 and NCI-H727), neuroblastoma (SHEP-TET), breast (M231), kidney (RCC10) and malignant pleural mesothelioma lines (H2795). While there was no toxicity to control healthy cells, TRAIL<sup>+</sup> exosomes were capable of triggering apoptosis in TRAIL-resistant cancer cells (132).

The TRAIL receptor binding to target cells, which activates the caspase cascade and results in death, was suggested as the therapeutic mechanism of TRAIL-MSC-EVs. Furthermore, TRAIL-MSC-EVs have exhibited therapeutic efficacy in TRAIL-resistant cancer cell lines, which is noteworthy (132). The study by Shamili *et al* (133) also demonstrated the anti-tumor activity of TRAIL-transfected MSC-derived exosomes in a mouse model of melanoma. Their findings suggested that when TRAIL-expressing exosomes were injected into mice, they delayed the appearance of tumors and attenuated tumor growth (133). Of note, they proposed that a combination of TRAIL-exosomes with another chemotherapeutic may be explored as a promising therapeutic tool (133). The

diagrammatic representation of how TRAIL-expressing exosomes would lead to cell death in recipient HCC cells is presented in Fig. 3. A list of studies demonstrating the exosomal delivery by TRAIL and its clinical significance is presented Table III.

Several studies have found a synergistic effect between TRAIL and sorafenib, suggesting that combined treatment with these agents may lead to the development of effective therapy for overcoming TRAIL resistance in cancer cells. For example, a previous study demonstrated the synergistic effects of sorafenib and TRAIL, where Sorafenib considerably increased the cytotoxicity TRAIL to HCC cells (134). The enhancement in cytotoxicity may be obtained from the down-regulation of anti-apoptotic proteins by sorafenib. Similarly, another study by Chen *et al* (135) revealed that sorafenib sensitized TRAIL-resistant HCC cells to TRAIL-induced apoptosis by inhibiting STAT3 (135).

Moreover, the application of Sorafenib enclosed within exosomes into the target site offers a number of benefits as opposed to oral administration. As depicted by a previous study, exosome-encapsulated DOX delivery increased the therapeutic index in breast and ovarian cancer mouse models compared to exosome-free DOX (124). It was also demonstrated by *in vitro* and *in vivo* experiments that exosomes loaded with DOX limited heart toxicity by partially reducing the passage of DOX through cardiac endothelial cells (124).

The major issue with the safe, specific and efficient delivery of miRNAs is their property of being easily degradable before reaching the target organ. The lipid bilayer membrane of EVs protects the enclosed miRNA, preventing it from degradation and facilitating its effective delivery to the target site (136). Previous research has demonstrated that exosomes can safely enclose and carry miRNA to target cells of multiple types of cancer. For example, Almanza *et al* (137) demonstrated that EVs containing miR-335 effectively and long-lastingly restored the endogenous miR-335 pool in human triple-negative breast cancer cells, suppressing the expression of the miR-335 target gene SOX4 transcription factor, and significantly reducing tumor development *in vivo*.

Previously, another group (138) reported utilizing EVs as a delivery agent for miR-335 both *in vivo* and *in vitro*. They were successful in demonstrating that the safe administration of fibroblast-derived EVs that were loaded with miR-195 may concentrate inside the tumor, reduce the size of tumors, and increase the longevity of treated rats in a rat model of cholangiocarcinoma (138). Wang *et al* (139) demonstrated that miR335-5p could be successfully supplied to hepatoma cells by utilizing exosomes as a delivery agent both *in vivo* and *in vitro*. They observed the progression of HCC cell development when stellate cells were co-cultured with HCC cells due to exosomal transfer and noted the downregulated expression of miR-335p in both cells and exosomes (139). The target genes identified in terms of HCC are CDC42, NRG1, EIF5, CDK2, EIF2C2, LIMK1, PLK2, RGS19, THBS1, YBX1 and TCF3. However, upregulating the expression of miR-335-5p in stellate cell-derived exosomes has been shown to restrict HCC cell proliferation and invasion *in vitro*, and cause tumor shrinkage in mouse models (139). A schematic diagram of the exosomal delivery of miR-335 into recipient HCC cells and the mode of action based on the aforementioned investigations is presented in Fig. 5.

Table III. List of recent studies on exosomal delivery of TRAIL for cancer treatment.

| Cancer types  | Donor cells                         | Results  | (Refs.) |
|---|-------------------------------------|--|---------|
| Melanoma and lymphoma   | K562 cells<br>(lymphoblasts)        | Induction of apoptosis in cancer cells and control tumor progression <i>in vivo</i>  | (131)   |
| Lung cancer ( <i>in vitro</i> ), pleural mesothelioma ( <i>in vitro</i> ), renal cancer ( <i>in vitro</i> ), breast adenocarcinoma ( <i>in vitro</i> ), neuroblastoma ( <i>in vitro</i> ) | MSCs                                | Highly efficient at selectively inducing apoptosis in cancer cells and TRAIL delivery by MSC-EVs at least partially overcomes TRAIL resistance in cancer cells                       | (132)   |
| Melanoma  | MSCs                                | Delay in the appearance of tumors and attenuation of tumor growth  | (133)   |
| Lymphoma  | Myeloid leukemia cells              | Increased apoptosis of leukemia cells  | (147)   |
| Human lung adenocarcinoma   | 293T cells                          | Dinaciclib and TRAIL exert synergistic effects on TRAIL-mediated apoptosis   | (148)   |
| Lung cancer   | MSCs                                | EV-encapsulated TRAIL and dinaciclib can overcome the drug-resistance of lung cancer cells and are highly efficient for inducing the apoptosis of the TRAIL-resistant A549 cell line | (149)   |
| Malignant melanoma  | RAW 264.7<br>(macrophage cell line) | TRAIL-Exo/triptolide improved tumor targetability, enhanced cellular uptake, inhibited the proliferation, invasion, and migration, and induced the apoptosis of A375 cells           | (150)   |

MSCs, mesenchymal stem cells; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DR, death receptor; EVs, extracellular vesicles.

It is also noteworthy that miR-335 modulates the sensitivity of sorafenib against HCC cells. As shown in the study by Kim *et al* (43), Siah E3 ubiquitin protein ligase 2 (SIAH2) is the target of miR-335, where miR-335 contributes to the sensitizing effect of anticancer drugs via the expression enhancement of histone deacetylase 3 (HDAC3). SIAH2 overexpression was shown to result in anticancer drug resistance due to its effect on HDAC3 expression and ubiquitination (43). These findings suggest that miR-335 may be a promising anticancer agent. When combined with sorafenib, encapsulating it in exosomes increases its sensitivity to the drug, thus enhancing the therapeutic efficacy and preventing degradation.

## 6. Development of exosome-based TRAIL + miR-335 therapy

Although exosome-based cancer therapy displays exceptional therapeutic potential, there are still a number of substantial challenges that need to be resolved in order for its use to be feasible in clinical applications. The technology of exosome mass production is not yet standardized. Although small-scale GMP exosome production has been shown to be viable, there are still numerous obstacles in large-scale production (121,140). Numerous companies are still struggling to produce exosome on mass scale level. However, few companies, such as CK Exogene, a Korean biotechnology firm, have managed to overcome the issue of low exosome yield and

have acquired the patented technology (10-2020-0062365) for exosome mass production (141) and this company is currently developing exosome-based anticancer drug for patients with liver cancer using the aforementioned candidates i.e., TRAIL and miR-335. A schematic overview of anticancer candidates, including dorafenib encapsulated in exosomes as a delivery agent is presented in Fig. 6).

To the best of our knowledge, the present review is the first of its kind, exploring and combining the cutting-edge feature of exosome with novel anticancer candidates (TRAIL and miR-335). The aim of the present review article was to present the compiled investigations of TRAIL and miR-335, both of which have been extensively explored in the past, along with the added benefit of utilizing exosomes as a carrier. Exosomes encapsulating TRAIL have the potential to overcome the challenging issue of resistance among HCC cells towards TRAIL-induced apoptosis, thus rendering TRAIL more effective in killing cancer cells. Such a strategy not only provides long-term and effective anticancer treatment for patients with HCC, but it has also been reported to overcome the issue of drug resistance, which is the major challenge to current drug therapy for liver cancer.

Additionally, to receive the successful outcome of any drug treatment, the accessibility of the drug to the target organ is imperative and necessitates the requirement of an effective delivery route. Even though drugs targeted against HCC comprise various delivery routes, including the direct

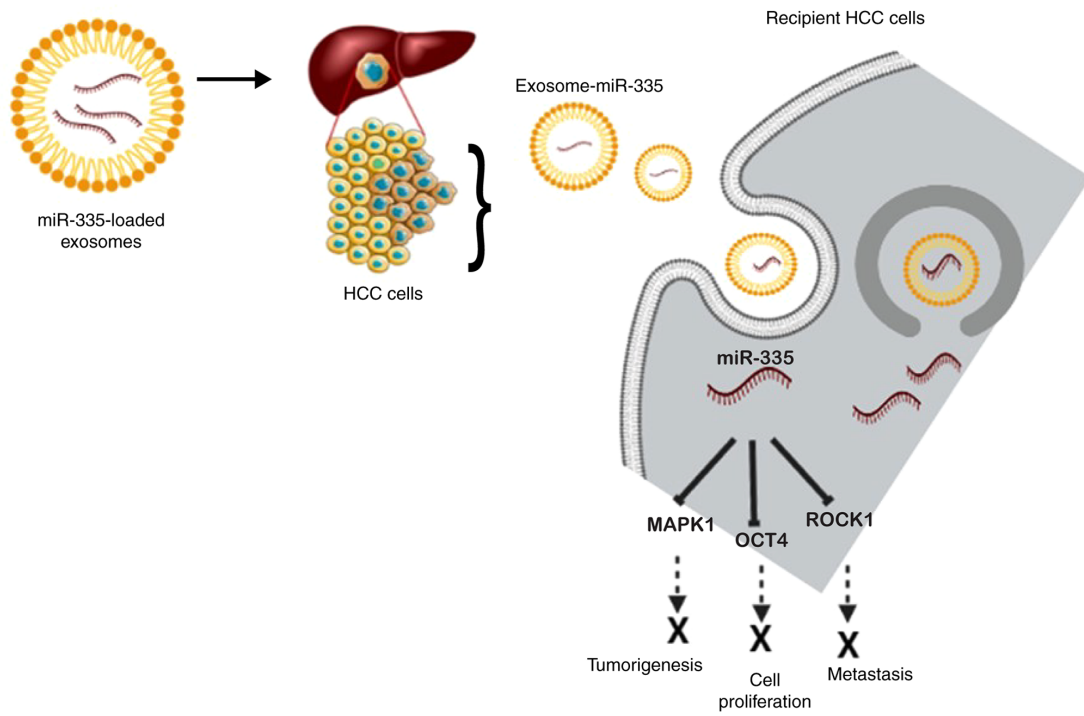


Figure 5. Graphical overview of the exosomal delivery of miR-335 into recipient HCC cells and mode of action. miR-335 inhibits the expression of MAPK1, OCT4 and ROCK1 to suppress the malignancy of cancer cells, inhibiting cell proliferation, metastasis, and tumorigenesis. The figure was created using BioRender.com. HCC, hepatocellular carcinoma; MAPK1, mitogen-activated protein kinase 1; OCT4, octamer-binding transcription factor 4; ROCK1, Rho-associated coiled-coil-containing protein kinase 1.

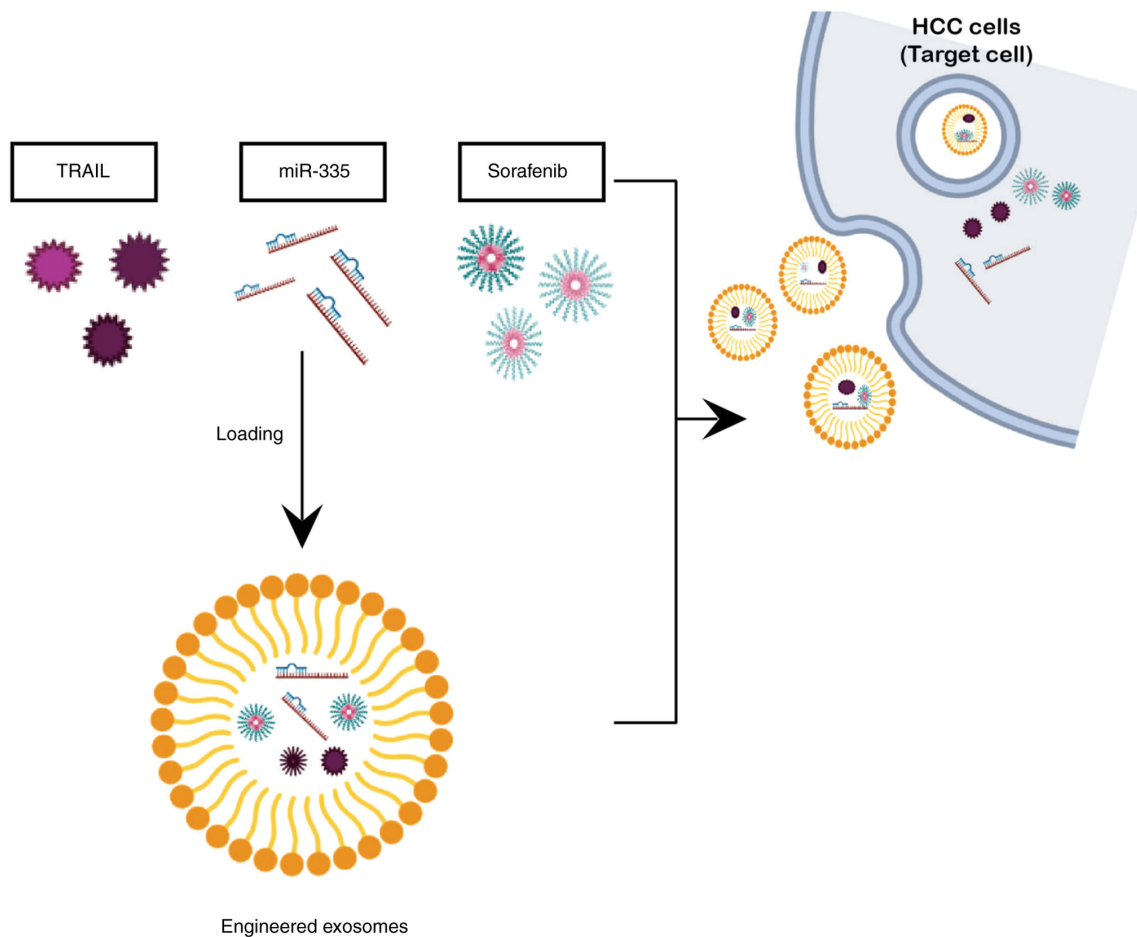


Figure 6. Overall strategy of exosome-based targeted therapy encapsulating TRAIL, miR-335 and sorafenib. The figure was created using BioRender.com. HCC, hepatocellular carcinoma.

injection into the liver, intra-arterial drug delivery is an effective technique for targeting the tumor site with multiple agents. Compared to intravenous delivery, intra-arterial drug administration expedites the systemic clearance and enhances the intra-tumor drug concentration (142). Owing to such high magnitude of benefits conferred by exosome-based TRAIL-miR-335 delivery from preventing metastasis to inducing cytotoxicity in cancer cells specifically, this approach has the prospects to be provided to patients with all stages of liver cancer from stages 0 to 4.

## 7. Conclusions

Exosomes offer the versatile characteristics of an efficient delivery system for both TRAIL and miR-335 as anticancer candidates. The incorporation of the benefits of exosomes, with them being a natural cellular carrier and the combination of these novel candidates with a standard drug, such as sorafenib would have a sensitizing effect and has been proven to yield a synergistic anti-cytotoxicity effect on TRAIL-resistant cancer cells. Moreover, this strategy also has the potential to overcome resistance to sorafenib, the most prevalent issue of the current drug treatment program among patients with HCC. The most prominent significance of exosome-based technology lies in the fact that this approach can be applied to all types of cancer and encompasses the benefit of overcoming drug resistance, which is the most prevalent issue in current drug treatment regimen. The present review thus provides an insight into the development of exosome-based therapy and the possibility of its bench-to-bed translation for providing an exceptional anticancer treatment.

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## Availability of data and materials

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## Authors' contributions

All authors (NT, YJC, KHY, TBW, DK, DC and JK) were involved in the drafting and revision of the manuscript, and in critically revising the manuscript for important intellectual content. All authors have read and approved the final manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The purification strategy for the mass production of highly purified and concentrated exosomes is subject to Korean patent application no. 10-2020-0062365, associated with CK-Exogene, Inc. JK and NT are employees of CK-Exogene, Inc. The other authors (YJC, KHY, TBW, DK and DC) are not associated with CK-Exogene, Inc. and declare that they have no competing interests.

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