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Stem cells-derived suicide gene exosomes: a promising platform for innovative cancer therapy

Invited review

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Commitment to the Legacy of Howard M. Temin on the 50th Anniversary of His Nobel Prize Recognition.

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Innovative cancer treatments are needed for metastatic tumors that currently do not have adequate therapies. This review highlights recent progress in suicide gene therapy using small extracellular vesicles, particularly exosomes, as a novel form of intracellular anticancer therapy. Suicide gene exosomes are produced by tumor-targeting human mesenchymal stem cells (MSC) that have been genetically modified to express the yeast cytosine deaminase::uracil phosphoribosyl transferase fused gene (*yCD::UPRT*) along with the prodrug 5-fluorocytosine (5-FC). The *yCD::UPRT*-MSC-secretome containing tumor-targeted exosomes converts 5-FC into the cytotoxic compound 5-fluorouracil (5-FU) and its metabolites within the tumor environment. The second popular system we are investigating involves the suicide gene exosomes derived from thymidine kinase of Herpes Simplex Virus in conjunction with a prodrug ganciclovir. Extracellular vesicles secreted by tumor-associated cells contribute to tumor growth and metastasis. When these cells are transduced with *yCD::UPRT* suicide gene, they can act as a source of therapeutic exosomes capable of intracellularly converting nontoxic prodrug 5-FC to a cytotoxic 5-FU. Combined action of suicide gene exosomes from MSCs and cancer-associated fibroblasts is a promising platform for aggressive tumor treatment. Furthermore, suicide gene exosomes can be enhanced with additional anticancer drugs and customized for targeted delivery. In this review, we trace the history of these findings, present therapeutic outcomes from *in vitro* and *in vivo* studies, and explore the future potential of therapeutically beneficial exosomes for cancer treatment.

Key words: gene-directed enzyme prodrug therapy, mesenchymal stem/stromal cells, suicide gene exosomes, cancer-associated fibroblasts

Although there have been significant advancements in standard cancer therapies, there is a pressing need for innovative treatment approaches for rapidly growing and highly aggressive tumors such as glioblastoma, pancreatic adenocarcinoma, metastatic melanoma, lung carcinomas, gastric carcinomas, and colon tumors, which have limited standard therapeutic options and generally low patient survival rates. Innovative cancer treatments must differ from conventional therapies, which primarily target rapidly dividing cells, often resulting in side effects and the development of drug resistance. The novel cancer therapies should specifically target tumor cells, tumor stem cells, and the tumor microenvironment, particularly cancer-associated fibroblasts (CAFs) that originate from mesenchymal stem/stromal cells (MSCs), as they play a crucial supportive role in the tumor microenvironment [1–3]. Moreover, it is essential that the novel therapies act within the cancer cells themselves, as this may help to prevent the development of chemo-radio-resistance and mitigate the side effects associated with standard treatments. MSCs play a key role in the repair of damaged tissues within organisms. This process occurs when MSCs migrate to the injury site and release various substances that, in a paracrine mode, perform multiple functions, such as promoting angiogenesis, preventing apoptotic cell death, modulating

the immune response, and initiating and sustaining regeneration processes in injured tissues. MSCs also localize to the tumor and contribute to the formation of the tumor stroma alongside other cells [4].

Extracellular vesicles (EVs), particularly those derived from MSCs, meet the stringent requirements for advanced cancer therapies. They effectively target cancer cells by penetrating cell membranes and being directed towards specific cell types, thereby minimizing off-target effects and associated side effects. Their potential for clinical application as agents for tissue regeneration, as well as anti-inflammatory and immunomodulatory treatments, has been extensively reviewed recently [5, 6].

In this review, we aimed to highlight the involvement of the retrovirus replication mechanism in cancer gene therapy. Our involvement in the B77 avian sarcoma retrovirus research began with a tumor that spontaneously developed in a hen named B77 [7]. Histopathological examination identified the cells as sarcoma cells, which were preserved through subcutaneous cell transplantation in chickens. Subsequently, it was discovered that only the cell-free extract was effective in inducing tumor growth in chickens [8]. There was an interest in determining whether the avian-derived B77 virus could induce tumor formation in mammals, particularly in rats. When chicken tumor cells were injected subcutaneously into newborn rats, a tumor designed RBI developed in a few of the rats. The avian B77 virus could be retrieved from rat cells by reinjecting them into chickens (Figure 1A) [9]. The presence of the virus in rat cells in a noninfectious state was termed virogenic [10], and we interpreted this finding with the assertion that "in rat cells, the information for B77 virus formation is integrated into the cell's genome" [11]. This statement prompted further investigation of the B77 virus in Howard M. Temin's laboratory, where he suggested a homology between RNA from Rous Sarcoma Virus (RSV) and the DNA of RSV-infected cells [12]. Since 1966, our research has focused on the role of retroviruses in carcinogenesis [13, 14]. The capacity of retroviruses to replicate within a host cell by converting their RNA genome into DNA, which subsequently integrates into the genomic DNA, a discovery for which Howard M. Temin was awarded the Nobel Prize, forms the foundation of all our gene therapy research [15]. This breakthrough initiated recombinant DNA technology and the creation of retroviral vectors. The modified retrovirus can integrate into genomic DNA and express genes within recipient cells, laying the groundwork for cancer suicide gene therapy [16].

Gene-directed enzyme prodrug therapy of cancer (GDEPT). The GDEPT, also known as suicide gene therapy, is accomplished by delivering a gene that encodes an enzyme to tumor cells, followed by the systemic administration of a nontoxic prodrug that locally induces apoptotic tumor cell death after enzymatic conversion into a cytotoxic agent. The experimental pipeline leading to the development of suicide gene-expressing exosomes from MSCs prepared from various tissues and the mechanism of their involvement in tumor cells is schematically depicted in Figure 1B. GDEPT is considered a promising approach for antitumor therapy [16]. The main emphasis has been on suicide genes, such as the thymidine kinase gene derived from the Herpes Simplex Virus (*tk-HSV*) and bacterial cytosine deaminase (CD) [17–20]. The prodrug ganciclovir (GCV) is activated by the thymidine kinase from the HSV, phosphorylating it into ganciclovir phosphate, which is then further processed by cellular enzymes into ganciclovir triphosphate, affecting DNA replication. The 5-fluorocytosine (5-FC) is converted by CD into 5-fluorouracil (5-FU). Positive outcomes from both GDEPT systems in animal studies have propelled the development of suicide gene therapy into multicenter clinical trials [21]. However, the disappointing results from the phase III, multicenter, randomized, controlled trial involving the *tk-HSV*/GCV system, which included 248 previously untreated glioblastoma multiforme patients, have led to the stop of several trials. The comparison between standard treatment, which includes surgical resection and radiotherapy, and adjuvant gene therapy utilizing *tk-HSV* fibroblasts revealed no significant difference [22].

Requirements needed for efficient GDEPT. The bystander effect is a phenomenon that plays a crucial role in enhancing the destruction of cancer cells beyond those that express the suicide gene, affecting nearby cells, which is vital for successful tumor ablation. In the CD/5-FC system, the low molecular weight of 5-FU and its metabolites allows for easy diffusion to adjacent cells [23]. On the other hand, the *tk-HSV*/GCV system relies on cellular contact that is established through gap junctions [24]. The source of CD has been recognized as a key factor influencing the conversion efficiency of 5-FC to 5-FU. Yeast CD produces a quantity of 5-FU that is 15 times greater than that generated by bacterial CD [25]. When uracil phosphoribosyl transferase is co-expressed with yeast CD through gene fusion, it enhances the effectiveness of 5-FC-mediated cell killing [26]. The creation of the bifunctional yeast cytosine deaminase::uracil phosphoribosyl transferase fused gene (*yCD::UPRT*) has been shown to improve the rate-limiting enzymatic processes involved in the conversion of 5-FC to 5-FU in adenovirus gene therapy, resulting in a 10,000-fold increase in the sensitivity of cells expressing the transgene to 5-FC [27].

The characteristics of a retroviral vector are crucial for the therapeutic outcome of GDEPT. Disappointing results from the preliminary clinical trials have highlighted the importance of cells utilized as vehicles for gene therapy. We have shown that adipose tissue-derived (AT-MSCs), along with MSCs from various sources such as bone marrow (BM-MSCs), dental pulp (DP-MSCs), umbilical cord (UC-MSCs), and placenta (PL-MSCs), can be transduced with suicide genes via retroviral infection. The mixed amphotropic and ecotropic retrovirus envelope glycoprotein is crucial for the successful infection of MSCs, leading to the

integration of a provirus into the host's genomic DNA. For the transduction of MSCs, we employed a retrovirus derived from a bicistronic retrovector that contains a suicide gene separated from a *neo* gene by an internal ribosome entry site. This configuration allows for the selection of a homogeneous population of transduced cells using the G418 antibiotic. It is vital to eliminate non-transduced cells to ensure the quality of the therapeutic cells, as these non-transduced MSCs can potentially support tumor cell growth through the secretion of cytokines, chemokines, growth factors, and exosomes. The concentration of G418 in the selection media varies depending on the cells used for transduction, typically ranging from 30 to 800 μg/ml, which can be predetermined. This process has been described in detail elsewhere [28]. The suicide gene is effectively expressed due to the strong retroviral promoters from the provirus integrated into the MSCs' DNA. We utilized the same vector design for the *yCD::UPRT*/5-FC and *tk-HSV*/GCV systems (Figure 1C).

MSCs as tumor-tropic vehicles for GDEPT. The recognition that adipose tissue is an abundant and easily accessible source of MSCs compared to bone marrow, where their content is much lower, has led to its preference as a gene delivery vehicle [29, 30]. The role of MSCs in the development of tumor stroma inspired us to develop AT-MSCs-mediated GDEPT. We have created two prodrug suicide gene therapy systems: MSCs engineered to express the fused gene *yCD::UPRT* with 5-FC as the prodrug (*yCD::UPRT*/5-FC system) [31], and MSCs expressing *tk-HSV* with GCV as the prodrug (*tk-HSC*/GCV system) [32]. In both approaches, the suicide genes are effectively expressed due to strong retroviral promoters derived from the provirus integrated into the cellular DNA. The MSCs modified with suicide genes retained their tumor-targeting capabilities. These cells have been referred to as therapeutic MSCs [33]. In preclinical translational studies, we observed significant growth inhibition of various human tumor types in nude mice following the intravenous administration of *yCD::UPRT*-MSCs, including melanoma [34] and prostate cancer [35]. The systemic administration of *yCD::UPRT*-MSCs resulted in therapeutic cell homing to subcutaneous tumors, thereby inhibiting tumor growth. However, the PCR analysis for *yCD::UPRT*-MSCs at the site of subcutaneously injected tumor cells the following day was unsuccessful, despite achieving an effective curative outcome [31]. Moreover, a positive therapeutic effect of both autologous and/or human *yCD::UPRT*-AT-MSCs was validated in the autochthonous prostate adenocarcinoma model in TRAMP mice, which spontaneously develop aggressive prostate cancer [36]. Additionally, the intracerebral application of *yCD::UPRT*-AT-MSCs in glioblastoma-bearing rats led to complete tumor regression in a significant manner [37, 38].

EVs facilitate communication between cells in the human body. All cells within the body release EVs, specifically exosomes, as a mechanism for intracellular communication. These vesicles transport their bioactive contents, which include crucial information in the form of mRNA, miRNA, and proteins, to distant cells. Furthermore, they serve as a means to eliminate waste or foreign molecules from the cells, thus preserving cellular homeostasis. Small EVs, known as exosomes, are heterogeneous particles that range in size from 30 to 150 nm and replicate the functions of the cells from which they are derived [39]. The secretome of MSCs, primarily composed of small EVs like exosomes, plays a vital role in paracrine communication between cells by conveying molecular information that leads to biological effects. Exosomes originating from MSCs are implicated in various regenerative processes in clinical applications, which have been recently reviewed in detail [5]. They exhibit a preference for tumors, maintaining the biological activity of their parent MSCs, and show comparable therapeutic potential. These exosomes are highly biocompatible, biodegradable, and exhibit low immunogenicity, making them ideal drug delivery vehicles that can effectively target cancer cells while reducing off-target effects and side effects [40]. MSCs-mediated therapy is evolving into a cell-free approach through the utilization of their nanoparticles. The primary advantage of exosomes over whole cells is the enhanced safety of the treatment. Unlike cells, EVs lack functional genomic DNA, do not replicate, and therefore cannot establish themselves in inappropriate locations where they might transform into potentially harmful cells under environmental influences.

MSCs transduced with a suicide gene secrete tumorotropic exosomes. Notable progress in MSC-based suicide gene therapy for cancer has arisen from our identification of *yCD::UPRT*-exosomes found in the conditioned medium (CM) of MSCs that have been modified to express this fused yeast gene. The exosomes associated with the suicide gene were generated from all human tissue-specific *yCD::UPRT*-MSCs. These exosomes carried mRNA of the suicide gene within their cargo. The release of exosomes containing the mRNA of the suicide gene enhances the understanding of the synergistic effects of the bystander effect and internalized exosomes [41]. Size-exclusion chromatography of the secretome derived from *yCD::UPRT*-MSCs revealed that the biological activity was notably concentrated within the exosome fractions (Figure 1D). The evaluation of tumor cell death caused by suicide gene exosomes containing mRNA of the *yCD::UPRT* gene was conducted in a dose-dependent manner. The growth of human prostate tumor cells PC3 caused by *yCD::UPRT* exosomes in the absence or presence of prodrug 5-FC is illustrated in Figure 1E. The human breast adenocarcinoma MDA-MB-231 cells were inhibited similarly (Figure 1F). The secreted *yCD::UPRT*-AT-MSCs suicide gene exosomes exhibited no alterations in size or heterogeneity when compared to exosomes released from parental cells, as illustrated in the images obtained from cryo-electron microscopy (Figure 1G).

The ability of exosomes released from *yCD::UPRT*-MSCs to conquer 5-FU resistance. The emergence of

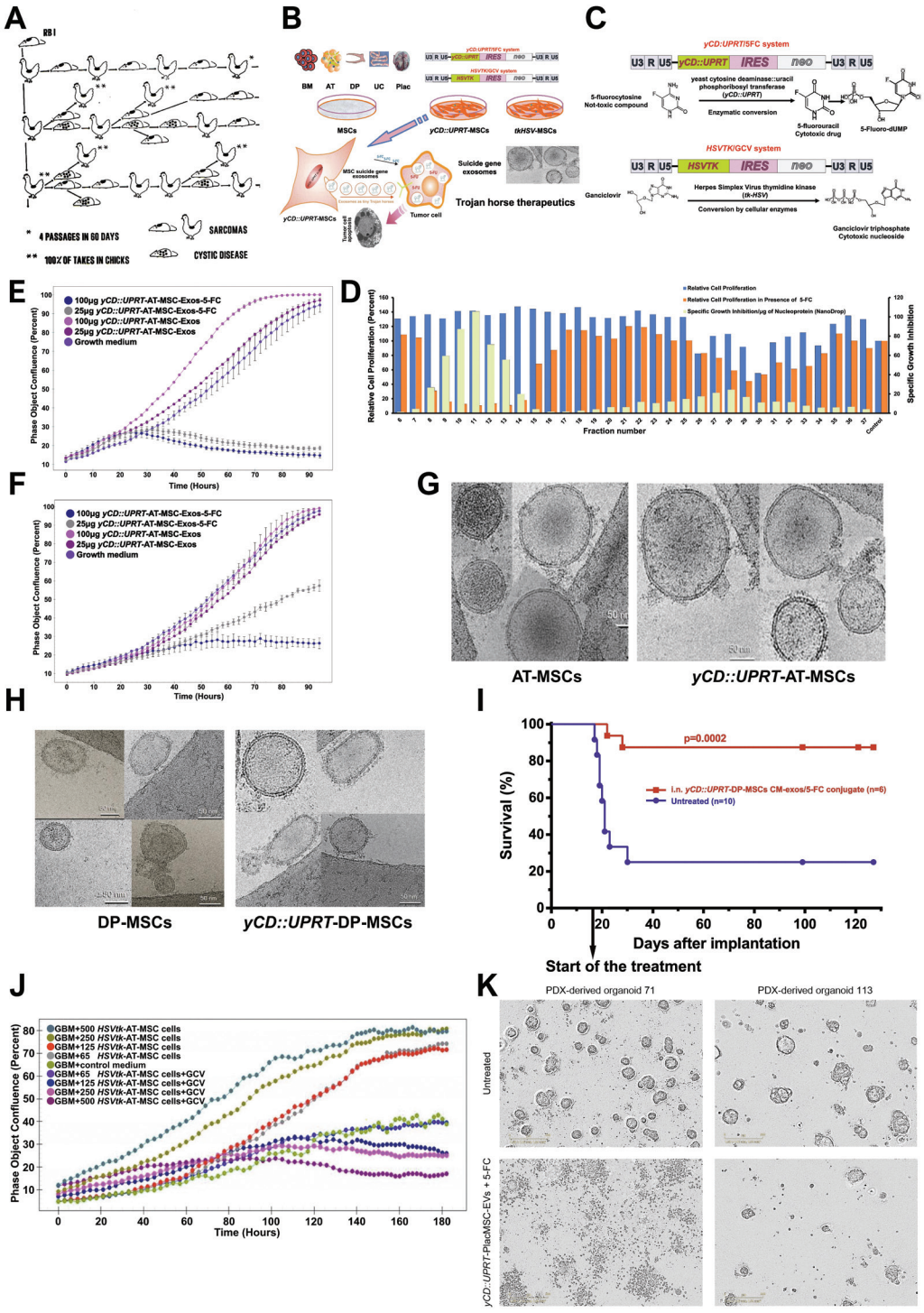

Figure 1. Gene-directed enzyme prodrug therapy of cancer mediated by exosomes. (A) Rescue of B77 virus from RBI rat tumors in chickens by cellular suspensions. (B) The schematic illustration of the pipeline leading to the development of suicide gene exosomes. (C) Scheme of retrovirus vectors used in our studies. (D)The activity of *yCD::UPRT*-MSC-exosomes in targeted tumor cells, which were separated using size-exclusion chromatography. Each fraction was evaluated with and without the presence of the prodrug 5-FC. (E) Dose-response of suicide gene exosomes tested in human prostate tumor cells PC3. (F) Dose-response analysis of suicide gene exosomes evaluated in human breast adenocarcinoma cells MDA-MB-231. (G) Cryo-EM images of exosomes derived from AT-MSCs compared to images of exosomes from *yCD::UPRT*-AT-MSCs. (H) Cryo-EM images of exosomes derived from DP-MSCs compared to images of exosomes secreted from *yCD::UPRT*-DP-MSCs. (I) Kaplan-Meier survival graph of C6 glioblastoma-bearing rats treated intranasally with *yCD::UPRT*-DP-MSC-exosomes conjugated with 5-FC. (J) The *tk-HSV*-AT-MSCs/ganciclovir system is efficient in killing of primary human glioblastoma cells. (K) The cytotoxic effects of *yCD::UPRT*-PlacMSC-cCM detected in primary PDAC-derived PDXO specimens.

chemotherapy-resistant tumor cells poses a significant obstacle to the effectiveness of cancer chemotherapy. It was reported that the incorporation of the *yCD::UPRT* transgene into 5-FU-resistant cells, which were derived from colorectal adenocarcinoma, had successfully overcome this chemoresistance [42]. Recently, it was reported that low miR-224-5p is involved in colorectal carcinoma resistance to 5-FU, as it is expressed at significantly lower levels in 5-FU-resistant cells compared to 5-FU-sensitive cells. This miRNA regulates the calcium-binding protein S100A4, which is upregulated in 5-FU-resistant colorectal carcinoma cells. Therefore, 5-FU combined with a calcium antagonist could be used as a therapeutic approach for 5-FU-resistant tumors [43].

Neurotropic character of dental pulp-derived MSCs and their exosomes. MSCs obtained from dental pulp, particularly from exfoliated deciduous teeth, exhibit distinct characteristics compared to MSCs derived from adipose tissue or bone marrow. Their MSCs-like immunophenotypic traits, elevated proliferation rates, capacity for multidirectional differentiation, and biological properties have been shown to surpass those of BM-MSCs and AT-MSCs [44]. These cells vary in the expression of pluripotent genes, primarily due to their embryonic neuroectodermal origin and characteristics [45]. A significant advantage of these stem cells over those sourced from other tissues is that they can be harvested through minimally invasive procedures, raising minimal ethical concerns. Furthermore, the neurotropic properties of DP-MSC-exosomes enable their application via intranasal routes. All the aforementioned attributes of human DP-MSCs remain intact when transduced with the suicide gene *yCD::UPRT*. When *yCD::UPRT*-DP-MSCs were tagged with iron sucrose, the secreted *yCD::UPRT*-exosomes administered intranasally migrated to intracerebral glioblastoma in rats [46]. Exosomes derived from naive and *yCD::UPRT* gene-transduced DP-MSCs showed no differences in size and heterogeneity compared to those secreted by the parental cells (Figure 1H). Exosomes containing the suicide gene from *yCD::UPRT*-DP-MSCs, when combined with the prodrug 5-FC, inhibited the proliferation of rat C6 glioblastoma cells and human primary glioblastoma cells *in vitro* in a dose-dependent manner. Upon repeated intranasal administration of these therapeutic exosomes, the growth of cerebral C6 glioblastomas in rats was suppressed, leading to the curation of a notable number of rats (Figure 1I) [47]. In agreement with the previously reported induction of protective immunity by tumors expressing the cytosine deaminase gene, which was eliminated with 5-FC, we observed the same effect [24]. *yCD::UPRT*-DP-MSC-exosomes induced immunity in cured rats against intracerebral reimplantation of glioblastoma cells [47].

Tumor cells derived exosomes as suicide gene Trojan horse therapeutics. Exosomes released by cancer cells demonstrate a range of biological activities. Studies on the composition and biogenesis of tumor exosomes indicate their ability to promote neoplastic growth, invasion, and metastasis by reprogramming recipient cells [48]. Additionally, exosomes from tumor cells mirror the traits of their originating cells, implying that when therapeutically altered, they may preferentially target tumors [49]. Exosomes from human lung, liver, and brain-targeting tumor cells are organ-specific and tend to infiltrate local cells at their predicted locations [50]. The uptake of tumor-derived exosomes by organ-specific cells aids in the establishment of the pre-metastatic niche [51]. For example, exosomes from pancreatic cancer have been demonstrated to initiate premetastatic niche formation in the liver [52]. Furthermore, exosomes from HeLa cells promote metastasis by inducing endoplasmic reticulum stress in endothelial cells [53]. In addition, tumor exosomes replicate the functions of their originating cells, enabling them to reach specific target cells in distant locations and organs [54]. These characteristics render tumor-derived exosomes promising candidates for suicide gene modification, paving the way for the creation of therapeutic vesicles that specifically target tumors and potentially metastases at the intracellular level [55]. In experiments, where we transduced various cancer cell lines with the *yCD::UPRT* gene through retrovirus infection, we found that secreted suicide gene exosomes functioned similarly to *yCD::UPRT*-MSCs [56]. They contained mRNA of the *yCD::UPRT* gene within their cargo. Upon internalization into recipient tumor cells, they triggered cell death through the intracellular conversion of 5-FC to 5-FU and 5-FUMP in a dose-dependent manner. Most tumor cell-derived suicide genes exosomes exhibited tumorotropic properties; when exposed to 5-FC, they effectively eliminated tumor cells while not affecting the proliferation of human skin fibroblasts and DP-MSCs *in vitro*. *In vivo* studies assessed the toxicity of conditioned media containing suicide gene exosomes alongside the 5-FC prodrug in a cohort of 16 C57BL/6 mice. The animals, which were injected 10 times over a 3-day period via both intravenous and intraperitoneal routes, displayed no signs of toxicity, such as weight loss, diminished general mobility, or any other behavioral changes throughout the 2-month observation period [56]. The tumor suicide gene exosomes facilitate the conversion of the prodrug into a cytotoxic agent within cancer cells upon internalization. These exosomes induce the death of various cancer cell types *in vitro*. Collectively, the results indicate that tumor cell-derived suicide gene exosomes may serve as cancer cell-targeted Trojan horses that produce cancer therapeutics within cancer cells.

Exosomes from MSCs transduced with HSV-thymidine kinase. The domain of suicide gene therapy was initiated by research in which *tk-HSV* was stably integrated into tumor cells [57]. AT-MSCs that were genetically modified to express the *tk-HSV* gene from a DNA retroviral provirus incorporated into cellular DNA inhibited the growth of human glioblastoma cell lines *in vitro* in the presence of the prodrug GCV [58]. Successful transfer of GCV triphos-

phate to tumor cells required cell contact, which was effectively achieved through gap junctions [59]. The retroviral vector used for MSCs transduction enables the generation of a uniform cell population of transduced cells through G418 antibiotic selection [28]. Since that time, suicide gene therapy has broadened to include various suicide gene systems for cancer treatment [60]. We transduced multiple MSCs derived from different human tissues with the *tk-HSV* recombinant retrovirus. The uniform population of *tk-HSV*-MSCs was observed to release exosomes containing mRNA of the suicide gene within their cargo. The *tk-HSV*-MSC-exosomes, in the presence of GCV, effectively induced cell death in glioma cell lines and primary human glioblastoma cells. The efficacy comparison revealed that 65 μg of exosomes (as protein) is equal to 65 *tk-HSV*-AT-MSCs (Figure 1J) [58].**Tumor-derived exosomes determine the dissemination of metastases to organs.** Each aggressive cancer type has a distinct metastatic pathway guided by secreted exosomes towards target organs. For instance, exosomes released by cutaneous melanoma facilitate the remodeling of lymph nodes and impair tumor immunity, leading to the formation of metastases in various organs [61, 62]. Conversely, uveal melanoma, despite sharing the same embryonic origin, exhibits a high incidence of metastases in the liver [63]. In general, EVs secreted by tumor cells and CAFs are organ-specific, initiating the formation of pre-metastatic niches [64]. Tumor cells maintain their intrinsic homing abilities to return to their original site. Fortunately, EVs secreted by tumor cells mimic the functions of their parent cells, allowing them to target appropriate cells in distant organs. These properties render tumor-derived EVs promising candidates for modifications aimed at developing innovative cancer therapies for preventing metastasis, such as personalized vaccine treatments [55]. It was reported that systemic administration of *yCD::UPRT*-MSCs and *HSVtk*-MSCs in combination with 5-FC and GCV inhibited the growth of human breast adenocarcinoma cells (MDA-MB-231/EGFP) that induce lung metastases in mice. Suicide gene exosomes were probably involved in the exerted synergic cytotoxic effect on systemically administered tumor cells in mouse lungs [65]. The potential prevention of metastasis formation may be particularly relevant in malignancies where the onset is delayed, such as uveal melanoma [66]. We have reviewed pieces of evidence regarding the possibility of using suicide gene *yCD::UPRT*-EVs derived from tumor cells, tumor stromal cells, and/or from several types of human MSCs as a novel therapeutic approach for metastases, via pre-metastatic niche modification. We believe that the neoadjuvant application of suicide gene *yCD::UPRT*-exosomes combined with the prodrug 5-FC may contribute to the prevention of metastatic dissemination [67].

Combined targeting of pancreatic ductal adenocarcinoma with suicide gene exosomes from MSCs and pancreatic CAFs. Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, with an increasing incidence worldwide. It is characterized by rapid progression and early metastasis. The prognosis of patients with advanced pancreatic cancer is extremely poor, despite the progress in integrative treatment. Novel therapeutic treatments are needed. In our PDAC preclinical study, we demonstrated that concentrated conditioned medium with suicide gene exosomes from placenta-derived MSCs, engineered to express *yCD::UPRT* enzyme, inhibits the growth of pancreatic cancer cells, and also their primary tumor microenvironmental partner, the pancreatic CAFs. We assessed the therapeutic potential of *yCD::UPRT*-Plac-MSC-cCM in PDXO models. The strong cytotoxic activity of suicide gene *yCD::UPRT*-Plac-MSC-exosomes was detected. The *yCD::UPRT*-Plac-MSC-EVs completely killed PDAC-derived PDXO specimens (Figure 1K). This has led to the identification of a novel cell-free GDEPT approach to pancreatic cancer [68].

Modification of MSC-exosomes with anticancer drugs. MSC-exosomes exhibit great potential in targeted tumor treatment due to their advantages of high stability, low immunogenicity, good biocompatibility, long circulation time, and homing characteristics; therefore, they are much safer than cells in therapeutic applications. MSC-exosomes containing anticancer drugs are directed towards specific cell types or tissues to achieve targeted drug delivery. There are several methods for preparing MSC-drug exosomes to carry noncoding RNAs or anticancer drugs. Therapeutic strategies involving artificially modified MSC-exosomes in tumor treatment have been thoroughly reviewed. These drug-laden MSC-exosomes are aimed at tumors, which enhances the local concentration of the therapeutic agent while reducing side effects [69–71]. In our research, human DP-MSCs were cultured with gemcitabine (GCB), resulting in its incorporation into MSC-exosomes that effectively inhibited the growth of pancreatic carcinoma cell lines *in vitro*. Additionally, we engineered *yCD::UPRT*-DP-MSC-exosomes with GCB, which exhibited an enhanced inhibitory effect on cancer cell growth [72].

Conclusion. Exosomes obtained from MSCs modified with suicide genes represent a promising new category of highly selective therapeutics aimed at cancer. The exosomes and/or soluble factors secreted by MSCs function in a paracrine or endocrine manner, performing roles akin to those of MSCs. The significant therapeutic potential of human *yCD::UPRT*-MSC and *tk-HSV* MSC-exosomes can be attributed to various factors. They are specifically targeted to tumors, which enhances the local concentration and thereby reduces off-target effects and side effects, acting intracellularly, circumventing the development of resistance, and exhibiting biocompatibility, biodegradability, and low immunogenicity. Furthermore, they can be modified for targeted delivery to specific cancer cells or engineered in conjunction with other drugs. We propose that MSC suicide gene exosomes may play a role in preventing metastasis when applied properly.

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