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<u>Review</u>

Engineered adult stem cells: A promising tool for anti-cancer therapy

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Abstract

Cancers are one of the most dreaded diseases in human history and have been targeted by numerous trials including surgery, chemotherapy, radiation therapy, and anti-cancer drugs. Adult stem cells (ASCs), which can regenerate tissues and repair damage, have emerged as leading therapeutic candidates due to their homing ability toward tumor foci. Stem cells can precisely target malicious tumors, thereby minimizing the toxicity of normal cells and unfavorable side effects. ASCs, such as mesenchymal stem cells (MSCs), neural stem cells (NSCs), and hematopoietic stem cells (HSCs), are powerful tools for delivering therapeutic agents to various primary and metastatic cancers. Engineered ASCs act as a bridge between the tumor sites and tumoricidal reagents, producing therapeutic substances such as exosomes, viruses, and anti-cancer proteins encoded by several suicide genes. This review focuses on various anti-cancer therapies implemented via ASCs and summarizes the recent treatment progress and shortcomings.

Abbreviation

ASCs: Adult stem cells

MSCs: Mesenchymal stem cells

NSCs: Neural stem cells

5   HSCs: Hematopoietic stem cells

HGF: Hepatocyte growth factor

PI3K: Ras-phosphoinositide 3-kinase

CD: Cytosine deaminase

5-FC: 5-fluorocytosine

10   5-FU: 5-fluorouracil

CEs: Carboxylesterases

HSV-TK: Herpes simplex virus thymidine kinase

GCV: Ganciclovir

OVs: Oncolytic viruses

15   HCC: Hepatocellular carcinoma

ASO: Antisense oligonucleotide

STAT3: Signal transducer and activator of transcription 3

IL-12: Interleukin 12

TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand

20   NP: Nanoparticle

AuNRs: Gold nanorods

TCRs: T cell receptors

CARs: Chimeric antigen receptors

MHC: Major histocompatibility complex

25   aPD-1: Anti-programmed death-1 antibodies

S-P-aPD-1: HSCs-platelet-aPD-1

PD-1: Programmed death-1

GvHD: Graft-versus-host disease

EV: Extracellular vesicles

30   yCD::UPRT: Yeast cytosine deaminase::uracil phosphoribosyltransferase

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Introduction

Cancer is one of the most lethal diseases that has been treated since the existence of human beings. Approximately 19.3 million new patients and nearly 10.0 million cancer deaths have been reported worldwide in 2020 (1). In the past few decades, before new therapies were

5 discovered, the combination of conventional treatments such as surgical resection, chemo- and radiation therapy were first-line treatments for many types of primary cancers. Although conventional methods are effective in the early stages of cancer, these treatments can cause adverse effects due to their non-selectivity (2). Lymphedema, which is the accumulation of lymph fluid in tissues, can be caused by damage to lymph nodes or blood vessels resulting from

10 surgery or radiation therapy (3). And chemotherapy drugs can cause neutropenia by targeting fast-growing cells such as healthy white blood cells (4). Therefore, studies on a variety of therapeutic interventions for improving the selectivity of conventional cancer treatment are in progress. On that note, numerous anticancer therapies and reagents have emerged and stem cell-mediated therapies using adult stem cells (ASCs) are considered attractive vehicles to

15 deliver anticancer reagents (Fig1). ASCs, such as mesenchymal stem cells (MSCs), neural stem cells (NSCs), and hematopoietic stem cells (HSCs), are undifferentiated populations that exist in our bodies, having the tumor tropic effect and the potential to partially replace conventional treatments (5).

An important characteristic of adult stem cells is their outstanding migratory and homing

20 ability (6). The migratory ability of ASCs initially elucidated in a xenograft mouse model with brain tumors (7). The exact mechanism of tumor tropism of ASCs is not fully elucidated, but several experimental evidence indicates that various chemokines such as monocyte chemoattractant protein-1, stromal cell-derived factor 1, and stem cell factor-1 are associated (8-11). The efficacy of NSC tropism has been reported not only in gliomas but also in other

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malignancies. It has been demonstrated that NSCs preferentially trace metastatic tumor foci in various mice organs. Intravenous injected NSCs or MSCs can migrate to metastatic nodes of breast cancer in the lung (12-16). Because of these abilities, adult stem cells have been actively studied as intermediate carriers of anticancer substances that track and kill cancer from the past

5   to the present.

1.   Stem cell-mediated therapies using MSCs and NSCs

1-1.   Prodrug – enzyme therapy

Using their migration ability to tumor sites, MSCs and NSCs are genetically engineered to

10   express therapeutic genes. The suicide gene cytosine deaminase (CD), found in *Escherichia coli* or yeast, converts the prodrug 5-fluorocytosine (5-FC) to the widely used cytotoxic anticancer drug 5-fluorouracil (5-FU) (17-19), which subsequently converts to the activate metabolites, inhibiting RNA and DNA synthesis and functioning of thymidylate synthase in the tumor (18). Cytosine deaminase expressing human MSCs, such as adipose tissue-derived

15   MSCs and a clonal immortalized NSC HB1.F3.CD, are both genetically engineered to express the CD suicide gene (20, 21). CD-NSCs have been especially studied in malignant gliomas as a first-generation enzyme/prodrug system to overcome difficulties associated with the delivery of anticancer compounds and the replacement of damaged tissues (22, 23). Research using yeast CD gene; yeast cytosine deaminase::uracil phosphoribosyltransferase (yCD::UPRT) has

20   been studied and applied in MSCs (24). Numerous *in vitro* and *in vivo* studies have extensively demonstrated the safety and therapeutic efficacy of expressing yCD::UPRT and *E. coli* CD gene (25-27). Additionally, a clinical trial using CD and 5-FC to treat high-grade gliomas has also been applied and completed (NCT01172964 and NCT04657315) (28).

Likewise, carboxylesterases are enzymes that alter the pro-drug CPT-11 (irinotecan) to its

active form SN-38 (7-ethyl-10-hydroxycamptothecin), a robust inhibitor of topoisomerase I (29-31). The pro-drug CPT-11 is currently being used in combination with other anti-cancer drugs for neuroblastoma and has progressed well in phase I clinical trial (32). The adenovirus-transduced HB1.F3.CD (human NSCs) with human enzyme is around 70-fold more efficient at converting to SN-38, than the endogenous wild-type human isoform of CEs in the liver (33, 34). The herpes simplex virus thymidine kinase (HSV-TK) is an enzyme that is widely studied for another suicide gene strategy, using the pro-drug ganciclovir (GCV) (35, 36). Applying the same transduced HB1.F3.CD, HSV-TK phosphorylates GCV into a monophosphate active form, which has the potential to effectively eliminate cancer (37).

1-2. Oncolytic virotherapy

Oncolytic viruses (OVs) preferentially target, replicate in, and eliminate cancer cells, rather than other normal cells (38). OVs can induce both innate and adaptive immunity (39). These characteristics have given new directions for anti-cancer therapy. Several oncolytic viruses have been tested in tumor-bearing animal models and have garnered major interest for scientists. Viral transduction of MSCs is frequently performed in adeno-, lenti-, and retro-viruses (40). The major obstacle of oncolytic virotherapy is viral destruction by host immunity. Several studies have shown that anti-viral destruction is mediated by endogenous interferon signaling (41). To overcome this limitation, MSC-mediated oncolytic virotherapy has emerged due to their tumor-tropic ability towards tumor foci and immunosuppressive functions (42, 43). MSCs produce several soluble factors and enzymes, such as cyclooxygenase pro-inflammatory cytokine interferon-γ (43). Previous studies have reported that MSCs specifically suppress immune cells, including the activity of T cells, B cells, and maturation of dendritic cells. These features provide robust shelters to safely deliver OVs to tumor sites (44). Until recently,

numerous researchers have attempted to improve the therapeutic efficacy of OVs. For example, Yoon AR et al. inserted Wnt-signaling inhibiting decoy acceptor sequences into an adenovirus, targeting hepatocellular carcinoma (HCC). This resulted in highly activating the Wnt signaling pathway and effectively destroying HCC. Additionally, reports indicate that MSC-mediated virotherapy prevents unpredictable side effects caused by MSCs due to the cells being lysed by adenovirus replication *in vivo* (45).

NSC-mediated virotherapy has been studied especially in gliomas and ovarian cancer. Aboody et al. reported that immortalized HB1.F3.CD21 (engineered neural stem cell line) in non-tumorigenic as well as enhancing tumor foci, inhibited the replication of adenovirus in glioma and ovarian cancer models. This oncolytic virus-loaded NSC model has currently progressed in a clinical trial of glioma patients (46). In addition, injection of NSCs with oncolytic adenovirus (NSC-CRAd-S-pk7) successfully distributes tumor area and allows the virus to deeper penetration into the tumor periphery (47).

1-3. Extracellular vesicle-mediated anti-cancer therapy

Extracellular vesicles (EV) are heterogeneous lipid bilayer particles that are produced from many types of cells including stem cells (48). Exosomes are extracellular membrane-bound vesicles ranging from 50-100 nm in diameter, comprising of protein, DNA, and RNA secreted by cells (49). As versatile therapeutic cargo, exosomes have several benefits. For instance, exosomes are involved in various biological processes such as antigen presentation, angiogenesis, and transferring horizontal miRNA (50). Alvarez-Erviti L et al. reported that in a mouse model, systemic injection of dendritic cell-derived exosome effectively delivered siRNA to the brain (51).

Unformulated antisense oligonucleotide (ASO) inhibitors have proven anticancer efficacy

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in multiple preclinical cancer models (52). ASO AZD9150 and CpG-STAT3ASO conjugates target the inhibition of oncogenic signal transducer and activator of transcription 3 (STAT3), which is an essential promoter in numerous human cancers (53). Adamus T et al. assessed the clinical efficacy of NSC-derived exosomes in the glioma as cargo for the delivery of oligonucleotides. Peritumorally administrated NSCs producing exosomes encapsulated CpG-STAT3ASO have demonstrated that NSC-mediated delivery significantly elevates the percentage of CpG-STAT3ASO and effectively reduces tumor growth, compared to the administration of oligonucleotides alone (54).

The previously mentioned CD/5-FC system was also used as a therapeutic reagent of the exosome. It has been reported that MSCs engineered to express the CD gene released exosomes contained CD mRNA. The exosomes were internalized by cancer cells and triggers apoptosis due to the conversion of prodrug 5-FC to active drug 5-FU (55). MSC-EVs carrying therapeutic substances such as miRNA were also evaluated to have anti-cancer effects. In a previous study, it was shown that miRNA-148-3p transferred by human umbilical cord-derived MSC leads to apoptosis by regulating tripartite motif 59 in MDA-MB-231 breast cancer xenografts (56).

1-4. Cytokine mediated therapy

Cytokines are small molecules normally produced by immune cells, involved in the regulation of immune responses, and comprise interleukins, chemokines, tumor necrosis factors, and lymphokines (57). In the mid-1990s, a high dose of interleukin 2 was found to be an effective anti-cancer agent, leading to the commencement of cytokine-based therapy (58). Since then, a variety of cytokines have been examined to evaluate anti-cancer efficacy. However, systemic administration of immunomodulating cytokines demonstrated several shortcomings. Because of the short half-lives of cytokines, administration of high doses of

cytokines can cause adverse side effects, including cardiac problems. Thus, ASCs are an appropriate model to deliver cytokines precisely to the tumor environment and prevent unfavorable side effects. IFN-β-inserted MSCs were the first cytokine-engineered MSCs, which inhibited the growth of melanoma *in vivo*. Also, it was reported for the first time, that

5   MSCs were positioned into the tumor and produced large quantities of IFN-β (59).

Subsequent studies confirmed that diverse cytokines and chemokines, such as the interleukin and interferon families, act as tumoricidal reagents when inserted in MSCs. Interleukin 7, interleukin 2, interleukin 12 (IL-12), interleukin 15, and interleukin 18 are reported as immunoregulatory cellular cytokines against cancer (60). Viral transduced MSCs

10   of IL-12 were capable of migrating to the tumor site, where the MSCs locally express the IL-12 gradually without disturbing other systems, thereby minimizing the inherent adverse side effects (61). MSCs overexpressing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) showed a 6-fold increased ability to kill tumor cells, as compared to the non-modified MSCs (12). MSCs expressing the death receptors TRAIL-R1 and TRAIL-R2 resulted in

15   TRAIL-induced apoptosis of tumor cells expressing the TRAIL death receptors (62). Additionally, a clinical study is conducted on therapeutic efficacy of TRAIL for patients. (NCT03298763)

1-5.   Nanoparticle-loadedoaded ASCs anti-cancer therapy

20   The use of nanoparticle (NP) carriers as a delivery system for selective anti-cancer agents attenuates unfavorable side effects and improves chemotherapeutic pharmacologic properties by altering the biodistribution and pharmacokinetics (63). ASCs such as MSCs and NSCs are considered appropriate vehicles to deliver therapeutic NPs to overcome drawbacks of traditional chemotherapy, such as suboptimal penetration and retention. Various cationic

polymers that destruct tumors chemically or physically as ASC-NP conjugated or in an internalized form have been widely studied (64). For instance, gold nanorods (AuNRs) improve several drawbacks, including damage to the surrounding healthy tissue due to the complex tumor geometries of photothermal therapy, i.e., heat generation when a tumor is exposed to

5 near-infrared radiation (65). Positioning the AuNRs within the tumor tissue converts the 810 nm nonharmful laser light into thermal energy, raising the local temperature to 28-60°C and leading to tumor resorption (66). To overcome this positioning issue, NSCs were selected to deliver AuNRs to the tumor foci. NSC-AuNR conjugates resulted in enhancing the tumor surrounding the ability of AuNRs, compared to administration of AuNRs alone, resulting in

10 improved anti-cancer efficacy (67). Mesoporous silica nanoparticles can transport drug to tumor site effectively, because of their surface pores. Anti-cancer drug can be released by certain stimuli such as pH, temperature, magnetic fields, and ultrasound (68, 69). It has been reported that mesoporous silica loaded MSCs release doxorubicin and lead apoptosis of cancer cells when the MSCs are stimulated by ultrasound (70).

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2. Stem cell-mediated therapies using HSCs

2-1. HSCs engineered with chimeric antigen receptors for anticancer therapy

HSCs are promising candidates for anticancer therapy, especially leukemia, through the transduction of genes that encode chimeric antigen receptors (CARs) or T cell receptors (TCRs).

20 CARs and TCRs recognize tumor-associated antigens directly, in a major histocompatibility complex (MHC)-independent manner (71). Although these methods have yielded unprecedented competency in B cell acute lymphoblastic leukemia, several challenges still need to be overcome, such as reduced T cell persistence and activity. Besides these, another concern associated with the use of mature T cells is the potential possibility for mispairing, i.e.,

a fusion of the introduced transgenic TCR chains with endogenous chains. Gene-editing technologies have enabled the elimination of the endogenous TCR and class-1 MHC-I to minimize graft-versus-host disease (GvHD) and allograft rejection (72). Another approach is the transplantation of HSCs. Using TCR- or CAR- transduced HSCs against cancers (especially leukemia) provides some advantages, which simultaneously resolves both problems of T cell persistence and TCR mismatching (73). First, the TCR- or CAR- transduced HSCs will constantly produce T-lymphocyte progenitors after transplantation, which thereafter undergo the natural T cell maturity process, developing potential immunity of the recipient. Next, unfavorable cytotoxicity of engineered TCR due to mispairing from transduced mature T cells are potently suppressed by HSCs that have not yet been rearranged (74). Giannoni F et al. reported that engraftment of transduced HSCs enhances the T cells expressing TCR recognizing tumor-associated antigen, which exerts specific anti-tumor activity [68].

2-2.   HSC-platelet conjugation-mediated therapy

Hu Q et al. reported that monoclonal anti-programmed death-1 antibodies (aPD-1) are secreted from the HSC-platelet-aPD-1 (S-P-aPD-1) cellular complex due to platelet activation. The immune checkpoint inhibitor aPD-1 blocks programmed death-1 (PD-1) expressed in various immune cells and disturbs the immunosuppressing ability of cancer through PD-1 ligands (PD-L1) (75). S-P-aPD-1 effectively promotes the delivery of aPD-1, and leukemia-specific T cells induce tumor growth suppression in the bone marrow, which is the site of acute myeloid leukemia (76).

Conclusion

ASCs are attractive models and give new insight into anti-cancer therapy, but the

application of ASCs will not completely replace previous traditional treatments such as surgery or radiation therapy. Stem cell therapy for cancer may be an attractive choice appropriated for patients diagnosed with metastasized malignancy, who have fewer treatment option than patients in the early stages. The Discovery of the tumor tropism of ASCs to the tumor has

5 resulted in the development of varied anti-cancer approaches. Of the various methods reported, engineered stem cell-mediated therapies have exerted anti-cancer efficacy not only in various primary cancers, but also in metastatic malignancies (77). As a therapeutic agent, engineered ASC-secreted materials such as oncolytic viruses, cytokines, and proteins exert anti-cancer activity, subsequently inducing the shrinkage of tumor volume via cancer cell death. Although

10 the outcome of ASC-mediated anti-cancer therapy is remarkable, several limitations and concerns about safety and shortcomings remain. As with other anti-cancer therapies, a single treatment of ASC therapies is difficult to completely eradicate the tumor and prevent tumor recurrence. Moreover, stem cells that migrate to the tumor site must remain only long enough to secrete the therapeutic agent, but the permanent alive of immortalized ASCs should be

15 avoided due to the possibility of them becoming tumorigenic. Portnow J et al. reported this based on first-in-human autopsy data, which stated that only infinitesimal NSCs remain longer than the required period of prodrug conversion. Additionally, it was reassuring to note that NSCs did not seem to be dividing (Proliferating cell nuclear antigen negative) and were positioned as single cells in the tumor (28). Another obstacle to cell-mediated therapy is

20 unequal biodistribution in the tumor foci after stem cell administration. Stem cells trace tumor sites through leaky vessels of the tumor. Depending on the size and type of tumors, varying degrees of blood vessel leakiness cause complications in the biodistribution and dose-response correlation. Moreover, engraftment of allogeneic immune cells and stem cells has the potential to possibly cause infections or GvHD (78, 79). To elucidate these concerns, increasing numbers

of preclinical and clinical researchers are developing and standardizing clinical trials. Advanced findings may guide investigators in the next step of stem cell therapies through cutting-edge technologies, and a better understanding of cancer physiology.

Conflict of interest statement

The authors do not have any conflicts of interest to declare.

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Figure Legend

Figure 1. Overview of the ASCs mediated anti-cancer treatments. ASCs mediated anti-cancer therapies are differentiated according to the use of MSCs, NSCs, and HSCs. Both MSCs and NSCs use the same carrier to deliver anti-cancer substances such as prodrug, oncolytic virus, exosomes, cytokines and photothermal heat. Viral transduced HSCs are localized in the bone marrow, and produce T cell progenitors that undergo normal immune cell maturation. Subsequently, immune cells expressing TCR that recognizes cancer-associated antigen, confer specific anti-cancer activity for a long time. Conjugation between HSCs and platelet adequately delivers immune check point inhibitors to the bone marrow, and augments an anti-leukemia immune response.
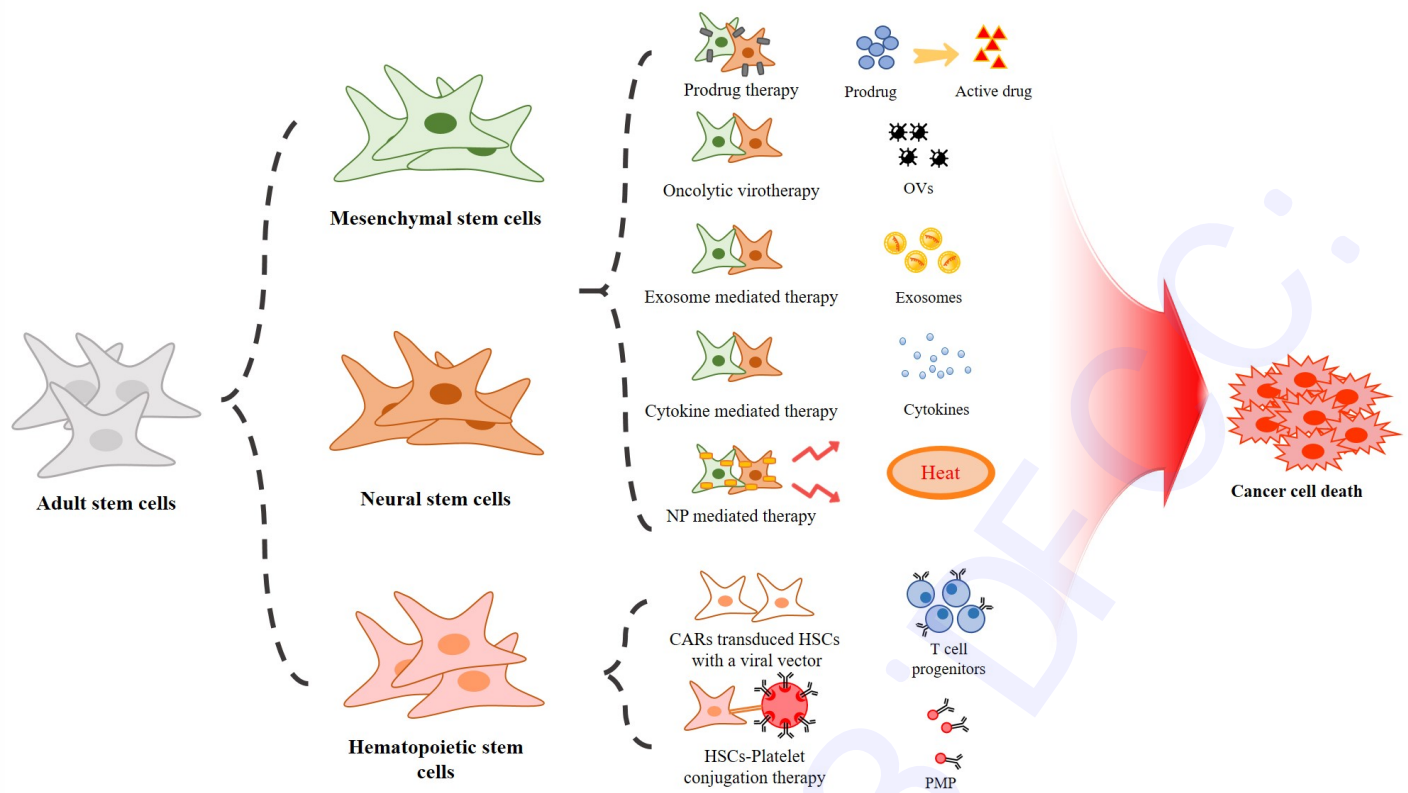
Fig. 1. Figure 1