

A promising therapeutic strategy for metastatic gestational trophoblastic disease: Engineered anticancer gene-expressing stem cells to selectively target choriocarcinoma (Review)

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Abstract. Gestational trophoblastic disease (GTD) is an unusual disease occurring in pregnancy that originates from abnormal trophoblastic cells and comprises a group of diseases with different properties of invasion, metastasis and recurrence. The GTD group includes hydatidiform moles and gestational trophoblastic neoplasms (GTNs), with GTNs being divided into invasive moles, choriocarcinoma, placental site trophoblastic tumors and epithelioid trophoblastic tumors. The present review focuses on current effective treatments for GTD, including conventional and novel promising direct enzyme prodrug therapies (DEPTs). Conventional therapies, such as chemotherapy and hysterectomy, are currently used in a clinical setting; however, the use of diverse DEPTs, including antibody-DEPT and gene-DEPT is also being attempted to cure GTNs. In addition, gene delivery tools using genetically engineered neural stem cells (NSCs) are presently being examined for the treatment of GTNs. The tumor-tropism of NSCs by chemoattractant factors is a unique characteristic of these cells and can serve as a vehicle to deliver anticancer agents. Previous studies have demonstrated that injection with NSC-expressing suicide genes into xenograft animal models has a significant inhibitory effect on tumor growth. Stem cells can be genetically engineered to express anticancer genes, which migrate to the metastatic sites and selectively target cancer cells, and are considered to effectively target metastatic GTNs. However, the safety issue of stem cell therapy, such as tumorigenesis, remains a challenge. Novel therapies comprising a combination of conventional and novel promising treatments

are anticipated to be definitive treatments for metastasized and/or recurrent patients with GTNs.

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1. Introduction

Gestational trophoblastic diseases (GTDs) are a group of disorders caused by the abnormal growth of trophoblast cells derived from placenta-forming tissues during pregnancy (1,2). GTDs can usually be diagnosed by ultrasound scans and blood tests during pregnancy (3). Malignant GTDs are diagnosed by an elevated level of β -human chorionic gonadotropin (hCG) (1,4). According to data from a study by the French Trophoblastic Disease Reference Center, ~30% of patients were initially diagnosed as having ectopic pregnancies, and 7 out of 18 patients initially received a misdiagnosis (5).

GTD is a term that includes benign and malignant tumors in this tissue. Hydatidiform moles are included in the benign GTD group upon clinicopathological classification. There are four types of malignant GTD; invasive moles, choriocarcinoma, placental site trophoblastic tumors and epithelioid trophoblastic tumors (6,7). Hydatidiform moles can occasionally progress to invasive moles or choriocarcinoma and spread rapidly. Choriocarcinoma, a malignant GTD, is a highly invasive tumor that is more likely to spread to other organs, including the lungs, liver and brain, through hematogenous routes (8,9). A previous study reported that the incidence of choriocarcinoma in pregnant women was approximately three to nine times higher in Asia than in Europe and North America (10,11). Compared with the hydatidiform mole, which accounts for 80% of all GTD cases, choriocarcinoma is relatively rare, and placental site

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trophoblastic tumors and epithelioid trophoblastic tumors are even less well known, and have been the subject of fewer studies (10,12).

According to the International Federation of Gynecology and Obstetrics (FIGO), the majority of cases of choriocarcinoma are chemotherapy-sensitive, with a survival rate of nearly 100% in low-risk groups and >80% in high-risk groups (13-15). There are four stages in FIGO staging system: stage 1 (only in the uterus), stage 2 (expansion into genital structures), stage 3 (extension of disease into lungs) and stage 4 (extension to the metastatic sites of the whole body) (15). Despite the fact that chemotherapy results in a significant cure rate, when cancer cells widely metastasize, the cure rate remains poor (16). Therefore, the development of effective therapies is the focus of present studies. In the present review, the characteristics of each type of GTD are presented, as well as the current strategies for GTD treatment, focusing on novel promising stem cell therapies.

2. Types of GTD

Hydatidiform mole. A hydatidiform mole is a disease caused by the atypical growth of normal trophoblastic cells, and is the most common benign lesion of the GTDs. The moles are classified into two types, complete hydatidiform moles and partial hydatidiform moles, according to morphological, histopathological and cytogenetic analysis (11). The treatment of the majority of patients afflicted with the two types of hydatidiform mole is focused on removal by dilation and curettage (17,18). There are two main risk factors for developing the moles: i) Maternal age; women ≤ 16 years or >40 years of age are 5 to 10 times more likely to develop hydatidiform moles than women aged 16-40 years; and ii) a previous history of a hydatidiform mole, which increases the incidence of another developing by ~1.8% (19).

Invasive moles. An invasive mole is a neoplasia that grows in the uterine wall; it can spread to other areas of the body, including the vagina, vulva and lungs, and usually occurs following conception in women of reproductive age (20,21). The most common signs of an invasive mole are prolonged vaginal bleeding and uterine enlargement. Invasive moles are clinically diagnosed by changes in hCG levels and are generally curable by extirpative procedures or hysterectomy (22).

Choriocarcinoma. Choriocarcinoma is a trophoblastic cancer that is liable to spread to multiple organs through hematogenous pathways. The most common symptom is vaginal bleeding, which also occurs in women with hydatidiform moles or during abnormal pregnancy (23). Choriocarcinoma is a rare cancer; however, the incidence of choriocarcinoma in Asian women is ~3 to 9 times higher than that in women in Europe and North America (9,24). Due to the high potential of vascular invasion, there are high risks of early metastasis to other organs, including the lungs, vagina, brain and liver (13,25). The majority of choriocarcinomas are treated with single-agent or combination chemotherapy depending on the severity, and the chemotherapy has been shown to exhibit a significant therapeutic effect (26). However, drug

resistance and systemic metastasis decrease the cure rate of chemotherapy (26).

Placental site trophoblastic tumors. A placental site trophoblastic tumor is a monophasic neoplasm GTD originating from extravillous trophoblasts (27,28); it is a benign lesion that develops from the placental implantation site and accounts for 0.25-5% of GTD cases globally (29). However, 10-15% of cases of this disease were reported to be clinically malignant tumors (30). Placental site trophoblastic tumors are a unique manifestation compared with other types of GTD, and this is due to several features: i) relatively low hCG serum levels; ii) late-onset metastasis; iii) slower growth; and iv) less sensitivity to chemotherapy (29). As the placental site trophoblastic tumor is generally resistant to chemotherapy, hysterectomy has been reported as an appropriate treatment (31).

Epithelioid trophoblastic tumors. An epithelioid trophoblastic tumor is a rare form of GTD. In 1998, it was identified as a distinct entity (32). It has been reported that this tumor commonly develops in fertile women with a history of gestational events, including molar pregnancy and spontaneous abortion, and that latency is between 2 months and 25 years (33). The incidence rate for the tumor is <2% among all GTDs (34). An epithelioid trophoblastic tumor is clinically similar to a placental site trophoblastic tumor as, it is resistant to chemotherapy and is slow growing (35). For patients with non-metastatic epithelioid trophoblastic tumors, a hysterectomy is recommended to maximize the therapeutic opportunity (34).

3. Current therapeutic methods and research for GTD

Malignant GTDs in the gestational trophoblastic neoplasm (GTN) classification include invasive moles, choriocarcinoma, placental site trophoblastic tumors and epithelioid trophoblastic tumors (26). Treatment of invasive moles and choriocarcinoma is principally associated with chemotherapy (36). The FIGO anatomical staging system is used to appraise the prognosis of patients and predict appropriate therapeutic strategies (15). Lung metastasis occurs in ~70% of patients with GTN, and brain metastasis occurs in 8-15% (37). The brain metastasis of a GTN is characterized by central necrosis and hemorrhage. Therefore, these patients are likely to present with neurological deterioration and intracerebral hemorrhage (ICH) (38). The probability of a cure rests on several prognostic factors, including age, pregnancy status, β -hCG concentration, extent of metastasis and tumor size (10).

Current treatments for GTD. One of the most effective remedies for hydatidiform moles is the termination of pregnancy (39-41). The termination of pregnancy diminishes the symptoms and prevents subsequent complications (42). Another possible treatment is chemotherapy. Methotrexate and dactinomycin are major chemotherapeutic drugs used in GTD (43). Hydatidiform moles have been successfully treated with methotrexate (44). In addition, the main treatment for invasive moles and choriocarcinoma is chemotherapy (36). The appropriate treatment for patients in the low-risk disease group is single-agent chemotherapy (36). The majority of patients with choriocarcinoma (~95%) caused by molar pregnancy

belong to this group, and treatment with methotrexate and dactinomycin alleviated the entire choriocarcinoma in 50-90% of patients (10,45). Of the total molar pregnancies, 80-85% of cases are benign without local relapse or metastasis to other organs. A total of 15-20% are invasive, and <5% are malignant with metastatic lesions (45). In the case of the high-risk disease group, combination chemotherapy is required instead of single-agent chemotherapy (46). A previous study showed that patients with recurrent malignant trophoblastic diseases also had a favorable prognosis with multiple-agent therapy, and 5-year survival rates were significantly increased (47).

Chemotherapy is regarded as the primary treatment for patients with GTN. However, patients with chemo-resistant or recurrent tumors require surgical therapy. Prior to performing resection, medical imaging tests are attempted in order to confirm the presence of lesions in the primary site or other organs. The majority of these tests are combined with chemotherapy to minimize metastatic potential by tissue manipulation during the treatment period. Furthermore, this combination therapy has the advantage of reducing the length of the hospitalization period and the number of chemotherapy cycles (37).

Treatment of GTD with direct enzyme prodrug therapy (DEPT). DEPT is defined as a therapy that converts pro-drugs to drugs at the desired location using artificially introduced enzymes (48,49). DEPT has the advantage of reducing the systemic toxicity of drugs by gaining active drugs only at a specific location (50). Due to this feature, a number of studies have been conducted to demonstrate the therapeutic effect of DEPT on choriocarcinoma.

Among the various types of DEPT, antibody-DEPT (ADEPT) exhibited antitumor effects in choriocarcinoma animal xenograft models (51). In ADEPT, antibodies designed against cancer antigens are connected to enzymes, and antibody-connected enzymes can selectively bind to cancer cells. Effective ADEPT should be able to produce long-term cytotoxicity in tumors linked with antibodies without serious toxicity to normal tissues (52). In a previous study using ADEPT, bacterial enzyme carboxypeptidase G2 exhibited a significant reduction of tumor growth resistance to conventional chemotherapy in a human choriocarcinoma xenograft model (51).

Gene-DEPT (GDEPT) is a method of selectively delivering genes that convert cytotoxic-prodrugs to drugs in tumor sites (53). The genes are selectively expressed in cancer cells by tumor-specific promoters or viral transfection. Weyel *et al* (54) confirmed that tumor growth in the choriocarcinoma xenograft model was inhibited by GDEPT using β -glucuronidase, which converts HMR 1826 to doxorubicin. GDEPT can be used to selectively target human malignancies, including GTNs, while reducing the adverse effects of biological drugs.

4. Gene therapy using genetically engineered neural stem cells (NSCs)

NSCs as a gene delivery tool for cancer treatment. NSCs are progenitor cells of the central nervous system (CNS) and are defined as self-renewing, multipotent cells that differentiate into neurons, astrocytes and oligodendrocytes (55). It has

been assumed that it would be feasible to develop alternative therapies for neurological diseases using the multipotential characteristics of NSCs (56). According to data from a studies published since 2000, NSCs were detected near the metastatic tumor when the stem cells were transplanted into a site remote from the brain neoplasia in animal models and found to be effective in delivering diverse therapeutic genes such as suicide genes and immunomodulatory genes to tumor foci (57-59). NSCs have several unique features: i) prolonged cell proliferation; ii) integration into the brain of the host without changing normal functions; and iii) migration toward neoplasms (60,61). Therefore, it is possible to target cancer cells by producing NSCs with chemotherapeutic properties (57,61).

Tumor-tropism of NSCs is indicated by chemoattractant factors produced in glioblastoma multiforme or normal tissue injured by tumor growth (62). To investigate this ability of the NSCs, the majority of studies were initially performed using intracranial glioma animal models, and their migration ability to various cancer types, including breast cancer, melanoma brain metastases, pancreatic cancer, neuroblastoma and lung cancer, was further confirmed (63,64). In a 2008 study, a correlation was identified between hypoxia and NSC migration ability (62). In glioma xenograft models, NSCs were distributed in hypoxic regions of intracranial tumors, and the expression of chemoattractant factors, including stromal cell-derived factor-1, vascular endothelial growth factor (VEGF) and urokinase-type plasminogen activator, were relatively decreased in hypoxia-inducible factor-1 α -knockdown cells (62). In addition, a variety of factors, including stem cell factor/c-kit system, VEGF/VEGF receptor, high mobility group box 1/receptor for advanced glycation end products, hepatocyte growth factor/c-met signaling, Annexin II and monocyte chemoattractant protein 1, were identified, which were associated with tumor-tropism of NSCs toward tumors (65). Based on the inherent migration ability of NSCs, stem cell therapy expressing anticancer genes has emerged as a promising therapy for metastatic cancer, including GTNs.

GDEPT and immunomodulatory gene therapy using NSCs. GDEPT using NSCs, a method that uses genetically engineered NSCs to express a gene encoding an enzyme that converts a non-cytotoxic or low cytotoxic prodrug to a cytotoxic metabolite, is a major approach for cancer therapy (66). These genetically engineered NSCs confer the following advantages on an effective cancer treatment: i) cancer-specific migration; ii) minimization of the risk of adverse drug events; and iii) increased treatment efficiency (61). Since NSCs have the capability for cancer selective migration, genetically engineered NSCs can deliver the anticancer genes at the tumor sites. Furthermore, Joo *et al* (67) reported that tumors were suppressed in the left and right hemispheres of the brain when genetically engineered NSCs were injected into the left hemisphere in brain metastasis animal models. This suggests that NSCs can migrate to the tumor-forming sites anywhere in the body and inhibit tumor growth. Cancer treatment using foreign enzymes expressed at the tumor site is an essential feature of GDEPT and can minimize the side effects when compared with conventional chemotherapy (57). The anticancer genes of GDEPT for humans typically use

Table I. Current and potential therapeutic methods for gestational trophoblastic disease.

Treatments	Cancer type	(Refs.)
Conventional therapy		
Surgical therapy		
Resection	GTN	(37,38)
Chemotherapy		
Methotrexate and/or dactinomycin	GTN	(43)
Methotrexate and EMA/CO ^a	Hydatidiform mole	(44)
DEPT		
ADEPT		
Carboxypeptidase G2	Choriocarcinoma	(51,52)
GDEPT		
β -glucuronidase	Choriocarcinoma	(54)
NSC therapy		
Carboxyl esterase	Lung cancer	(64)
CD and/or IFN- β	Breast cancer and choriocarcinoma	(63,78)
CD and HSV-1 thymidine kinase	Ovarian carcinoma	(70)
Interleukin 12	Glioma	(80)
Interleukin 23	Glioma	(81)

^aMulti-agent chemotherapy, including etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine. GTN, gestational trophoblastic neoplasm; DEPT, direct enzyme prodrug therapies; ADEPT, antibody-DEPT; GDEPT, gene-DEPT; NSC, neural stem cell; IFN- β , interferon- β ; CD, cytosine deaminase; HSV-1, Herpes Simplex Virus type 1.

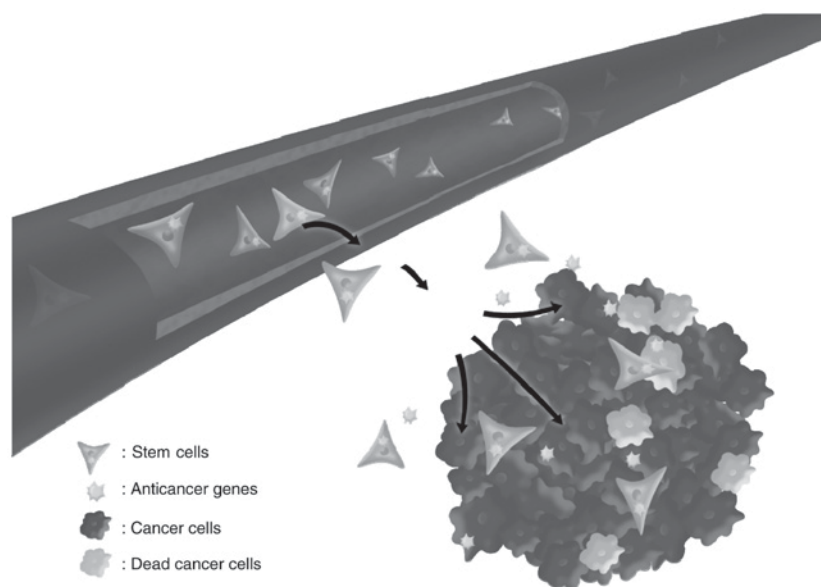


Figure 1. Schematic diagram illustrating how stem cells target disseminated tumors. Genetically engineered neural stem cells express anticancer genes targeted to tumors spread throughout the whole body, specifically inhibiting the cancer cells at the tumor site.

those originated from other species in order to minimize the pro-drug activity of endogenous enzymes (68). One example of stem cells expressing such genes is HB1.F3.CD cells, which are genetically engineered NSCs expressing the *Escherichia coli* (*E. coli*) cytosine deaminase (CD) gene. HB1.F3.CD cells were genetically engineered from fetal telencephalon-derived

HB1.F3 cells (69). The CD gene originating in *E. coli* converts non-cytotoxic agent 5-fluorocytosine (5-FC) to cytotoxic agent 5-fluorouracil (5-FU) (70). *E. coli* CD is an enzyme from the pyrimidine salvage pathway that deaminates the anti-fungal drug to 5-FU (71). Compared with direct 5-FU chemotherapy, the CD/5-FC approach may be clinically beneficial due to the

unique property of 5-FC, in that it can cross the blood brain barrier (72,73). Numerous studies demonstrated that HB1.F3.CD cells expressing the CD gene significantly inhibited the proliferation of cancer cells and suppressed tumor growth in the presence of 5-FC (63,69,74-76).

Treatment of sickness by enhancing or diminishing the immune response is termed immunotherapy. The use of cytokine genes to increase the antitumor response is particularly effective in cancer treatment. A previous study by Panelli and Marincola identified that 9% of renal cell carcinoma and 7% of melanoma patients were cured when treated with high concentrations of interleukin (IL)-2 in a total of 283 patients (77). Thus, NSCs engineered to express immunomodulatory genes, including interferon (IFN)- β , IL-4, IL-12 and IL-23, can effectively treat cancer by expressing them at the tumor site (57). For example, in mouse xenograft models with human colorectal cancer, tumors of mice injected with NSCs expressing CD alone (HB1.F3.CD) exhibited a 56% reduction of tumor volume compared with the control, while those of mice injected with NSCs expressing CD and IFN- β (HB1.F3.CD.IFN- β) exhibited a reduction of ~76% (75). Furthermore, treatment with these stem cells in choriocarcinoma metastasis or xenograft models inhibited tumor growth and decreased metastasis (78). Kim *et al* (78) reported that the volume of the choriocarcinoma tumor was smaller by approximately half compared with that of the control groups when using therapeutic genes in a choriocarcinoma xenograft model, and that the lung metastatic area of the HB1.F3.CD.IFN- β group was reduced by ~45% compared with that of the HB1.F3 group in a metastasis model.

However, stem cell-based gene therapy has two major challenges to overcome. The first challenge is the safety of the therapy. When human stem cells remain stable in the body, the cells can cause genetic and epigenetic alterations (79). Furthermore, undifferentiated embryonic stem cells are likely to form teratocarcinoma (79). The second challenge is the immune response. The vector that introduces the gene has the potential to induce an immune response, and non-autologous stem cells will result in immunological rejection (79). These issues are obstacles preventing phase III clinical trials of stem cell-based gene therapy from being conducted, and further studies should be performed in order to address them.

Overall, NSC therapy is a promising strategy as it is suitable as a tool for delivering anticancer genes to the metastatic sites of the body and it has been proven to have significant therapeutic effects. However, additional research is essential for the development of a stable and effective therapeutic method.

5. Conclusions

In the present review, conventional therapeutic tools and novel research fields for treating GTD were investigated and these are summarized in Table I. Chemotherapy, a major treatment for GTNs, has already achieved a significant cure rate. Depending on the extent of metastasis determined by FIGO stage, chemotherapy with a single or multiple agents is used; however, there is a possibility of systemic toxicity and recurrence. Furthermore, chemotherapy has limited effects on

recurrent tumors due to their chemical resistance. It is imperative to overcome these problems in order to effectively treat the tumors at the metastatic site.

Previous studies have demonstrated that NSCs have the ability to migrate to the metastatic regions of the whole body, as demonstrated by studies in which NSC injected in the left hemisphere suppressed tumors in the right hemisphere. The intrinsic properties of these cells are expected to be clinically useful for the targeted delivery of therapeutic agents to tumors that have spread throughout the body. However, the number of NSCs that can reach tumor nodules located at a considerable distance from the injected site will likely be limited. Therefore, studies of NSC-based therapy should maintain or improve the intrinsic tumor tropisms of NSCs.

As a result of the cancer-specific migration effect, NSCs are better suited as a therapeutic delivery tool for metastatic tumors than other cell types. The CD/5-FC approach with NSCs has a tremendous advantage in the field of drug delivery for cancer treatment. Furthermore, NSCs expressing several therapeutic genes, e.g. CD-expressing NSCs in combination with IFN- β , exhibited synergism of tumor suppression. Thus, genetically engineered stem cells expressing anticancer genes migrate to the metastatic sites and selectively target cancer cells (Fig. 1). Although chemotherapy for GTNs has considerable cure rates, combination with novel therapies is required to ensure definitive treatment of patients with metastasis and recurrence.

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Availability of data and materials

Not applicable.

Authors' contributions

GSK and KCC were involved in the study conception and design. GSK collected and assembled data; prepared the figure and table. GSK and KAH wrote the manuscript, and KCC reviewed literature. GSK and KAH ensured that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. GSK, KAH and KCC agreed to be accountable for all aspects of the work, and KCC provided final approval of the manuscript to be published.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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