Prodrug Gene Therapy for Cancer Mediated by Mesenchymal Stem/Stromal Cells Engineered to Express Yeast Cytosinedeaminase::Uracilphosphoribosyltransferase

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Abstract
Prodrug cancer gene therapy mediated by human adipose tissue-derived mesenchymal stem/stromal cells (MSCs) engineered to express fused yeast cytosine deaminase::uracil phosphoribosyltransferase (Therapeutic Stem/Stromal Cells-ThSC) is an efficient experimental therapeutic modality for cancers. The attractiveness of prodrug cancer gene therapy by stem/stromal cells targeted to tumors lies in activating the nontoxic prodrug 5-fluorocytosine directly within the tumor mass to 5-fluorouracil, thus avoiding systemic toxicity. Prodrug administration not only eliminates tumor cells, but consequently kills the more resistant ThSC as well. The therapeutic potential of this system is universal and quite effective. In a series of papers, it was shown its effectiveness in targeting and killing human colorectal, melanoma, glioblastoma, colon, breast and bladder carcinoma cells both in vitro, and in vivo. Pilot preclinical studies demonstrated that intravenously administered ThSC were effective in significantly inhibiting subcutaneous xenografts bone metastatic prostate cells. Similar inhibiting effects were seen on spontaneous aggressive prostate adenocarcinoma on TRAMP mice. Complete tumor regression was observed in a rat glioblastoma intracerebral model. It is assumed that curative glioblastoma therapy is a consequence of elimination of glioma stem cells that drive the tumor progression. Both vector composition and unique properties of the vehicle stem/stromal cells contribute to high therapeutic efficiency. Mesenchymal stem/stromal cells transduced stably with yCD::UPRT gene produced exosomes. The exosomes upon easy internalization to tumor cells in the presence of 5-FC inhibit growth of a broad kind of cancer cells in vitro. The exosomes released from therapeutic stem/stromal cells significantly contribute to the therapeutic efficiency of the yCD::UPRT-MSCs/5-FC system. Results support arguments for beginning clinical studies for the treatment of high grade tumors and metastases.

Keywords: Prodrug cancer gene therapy; Mesenchymal stem/stromal cell; Yeast cytosinedeaminase::uracilphosphoribosyltransferase; Cell therapy

Introduction
Despite improvement in standard therapies, mortality caused by most solid cancers has not changed substantially over the last twenty years. Partially responsible for these failures are the lack of radiotherapy specificity, cytotoxic chemotherapy, the emergence of drug resistant cell populations and severe side effects. All cancers are thought to contain a small proportion of self-renewing tumor-initiating stem cells (either cancer stem cells, or proliferating cancer stem-like cells), that are responsible for tumor initiation and progression [1-4]. Standard cancer therapies such as radiotherapy, cytotoxic chemotherapy and biological therapy of solid tumors do not eliminate cancer stem cells, and may even select for more aggressive tumor stem-like cells due to induction of additional mutations [5,6].

Further progress in cancer therapy, against aggressive tumors and metastases requires novel therapeutic modalities able to attack cancer stem cells and/or kill tumor cells while avoiding systemic toxicity. Prodrug cancer suicide gene therapy directed to the tumor site by mesenchymal stem/stromal cells (MSCs) may be one possibility [7].

MSCs repair damaged tissues in organisms. This is accomplished as MSCs migrate to the site of injury and secrete a variety factors capable of a number of functions inducing and supporting regenerative processes in damaged tissues, inducing angiogenesis, protecting cells from apoptotic cell death and modulating the immune system. The tumor, being a "wound that does not heal", attracts MSCs [8]. MSCs home on the tumor and, together with other cells, form the tumor stroma [9].

The MSCs tumor tropism is the basis for therapies using them as a vehicle for delivery of therapeutic agents to the site of neoplasm. We have developed a prodrug gene therapy method mediated with MSCs that were engineered to express fused yeast cytosine deaminase::uracil phosphoribosyltransferase (yCD::UPRT) [10]. In a pilot study we showed that yCD::UPRT-AT-MSCs in combination with 5-FC augmented the bystander effect and selective cytotoxicity on target human colorectal HT-29 cells in direct co-culture in vitro. Directed migration ability of AT-MSCs and ThSC toward HT-29 tumor cells both in vitro and in vivo was confirmed. Moreover, significant inhibition of s.c. tumor xenograft growth by s.c. or i.v. administration was achieved by ThSC in immunocompromised mice treated with 5-FC [10]. The attractiveness of prodrug cancer gene therapy targeted by MSCs to tumors lies in the activation of the prodrug directly within the tumor mass, thus avoiding systemic toxicity [11,12].

We have shown that human adipose tissue-derived AT-MSCs as well as MSCs derived from various other tissues including bone marrow (BM-MSCs), dental pulp, umbilical cord and menstrual
blood derived endometrial regenerative cells can be transduced with yCD::UPRT by retroviral infection. Transduced cells can convert nontoxic 5-fluorocytosine (5-FC) to the effective cytotoxic compound 5-fluorouracil (5-FU). Vector construction allows for antibiotic selection of the transduced cells yielding pure populations of transduced cells [10]. Elimination of non-transduced cells is important because of potential tumor cell growth support cytokines, chemokines, growth factors, and exosomes secreted from the MSCs. The yCD::UPRT- transduced human adipose tissue (yCD-AT-MSCs) designated “therapeutic stem/stromal cells” (ThSC) had sustained tumor-tropic properties [13]. ThSC are prepared in vitro, with the yCD::UPRT gene integrated into the cells as a DNA provirus. The advantage of ThSC is the stable and effective production of the prodrug-converting enzyme under the control of a strong retroviral promoter. The ThSC are safe, because prodrug administration not only eliminates tumor cells, but consequently kills the more resistant ThSCs as well, thereby eliminating genetically modified cells from the host. Compared with conventional chemotherapy, yCD::UPRT/5-FC suicide gene therapy mediated by MSCs exhibited no significant systemic adverse effects.

The therapeutic potential of the MSCs-driven yCD::UPRT/5-FC suicide gene therapy system is universal and quite effective. ThSC effectively delivered the yCD::UPRT transgene to the site of tumor formation and mediated strong antitumor effects in vivo [9]. The universality of the therapeutic ability of expanded yCD::UPRT-AT-MSCs in presence of 5-FU was by the induction of strong bystander cytotoxic effects directed against human melanoma, glioblastoma, colon, breast and bladder carcinoma in vitro. Systemic ThSC administration resulted in therapeutic cell homing into subcutaneous melanomas and mediated tumor growth inhibition [14]. Specific tumor-tropic and tumor killing properties of ThSC have been demonstrated both by the negative therapeutic outcome of similarly transduced human fibroblasts and by systemic administration of 5-fluorouracil (5-FU), which was inefficient in inhibiting tumor growth (Figure 1).

yCD::UPRT-AT-MSCs/5-FC system inhibits human prostate cancer in animal models

Human prostate cancer (PC) is the second highest cause of tumor-related deaths of men in the western world. Clinically localized PC can be successfully treated by radical prostatectomy or radiotherapy. In contrast, advanced stages of the tumor, which are initially responsive to androgen ablation therapy, develop a phenotype refractory to therapy within a few years [15,16]. The most successful attempts to arrest this stage of the disease result in only a few months of prolonged survival [17]. Novel therapeutic modalities able to attack metastatic prostate carcinoma would be of great clinical interest.

Pilot preclinical study with nude mice, we demonstrated that yCD::UPRT-AT-MSCs were effective in significantly inhibiting subcutaneous xenografts of human bone metastatic prostate cells. The ThSC were administered i.v. with systemic delivery of 5-FC. Tumor regression by therapeutic stem/stromal cells was dose dependent and repeated applications improved the therapeutic outcome [18].

Recently, positive therapeutic effects of autologous and/or human yCD::UPRT-AT-MSCs cells were proven in the autochthonous prostate adenocarcinoma in TRAMP mice, which spontaneously develop aggressive prostate cancer. Intravenous administration of ThSC inhibited growth of autochthonous prostate carcinoma in a dose dependent manner [19]. These data strongly support the testing of yCD::UPRT-AT-MSCs in clinical studies of patients with metastatic prostate cancer.

yCD::UPRT-AT-MSCs/5-FC system in a simulated clinical therapeutic scenario induced complete regression of rat glioblastoma

Glioblastoma multiforme is the most frequent and lethal human intracranial tumor. The median survival for patients diagnosed with WHO grade III and grade IV glioma is only 8 to 15 months. The prognosis for recurrent malignant glioma with present therapies is poor, with a median survival time of 3 to 9 months. Glioblastoma is usually fatal within a year of diagnosis. The highly invasive character of glioblastoma cells, together with extensive neovascularization of tumor tissue and dispersion of tumor cells deep into surrounding normal tissue can be partially blamed for the failure of standard glioblastoma therapy modalities. Furthermore, the presence of glioma stem cells (GSCs) in the main tumor mass can contribute to therapeutic failure. GSCs, residing within a perivascular niche, initiate glioblastoma and are responsible for tumor progression [20,21]. GSCs possess stem cells characteristics [22,23]. They multiply only rarely by asymmetric cell division, are resistant to toxic agents and are therefore resistant to cytotoxic therapies requiring cell division [24]. Thus, the effective treatment of glioblastoma must include the challenging task of killing of tumor cells present in the tumor mass, disseminated tumor cells in brain tissue, and tumor initiating GSCs.

In a preclinical study, we tested the feasibility and efficacy of using human yCD::UPRT-AT-MSCs to treat an intracranial rat C6 glioblastoma known to be composed from glioma stem cells [25,26]. The experiments were designed to simulate conditions of future clinical application for high-grade glioblastoma therapy by direct injections of therapeutic stem/stromal cells into the tumor. Genetically modified therapeutic stem cells continued to exhibit tumor tropism when injected into a distant intracranial site and effectively inhibited glioblastoma growth after 5-fluorocytosine (5-FC) therapy in a ThSC dose-dependent manner (Figure 2).

The therapy led to complete tumor regression in a significant number of animals. Continuous intracerebroventricular delivery of 5-FC using an osmotic pump reduced the dose of prodrug required for the same therapeutic effect, and repeated administration of therapeutic stem/stromal cells increased survival time [13]. To simulate the usual steps human glioblastoma therapy, an intracerebrally grown glioblastoma was treated by partial resection with subsequent single or repeated intracerebral inoculations of ThSC cells, followed by continuous intracerebroventricular delivery of 5-FC using an osmotic pump.
pump simulating human therapy by an intraventricular catheter system (Ommaya reservoir). This therapeutic arrangement leads to curative therapy in a significant number of animals. The therapeutic outcome was dependent on the number of the ThSCs and was significantly better when stem/stromal cells were applied repeatedly. The CDy-BM-MSCs and CDy-AT-MSCs did not differ in the therapeutic efficacy, both kinds of cells led to curative therapy [7]. Intracerebral injection of therapeutic stem/stromal cells and 5-FC treatment did not result in any detectable adverse effects. Results support the argument for initiating clinical studies for treatment of high-grade brain tumors.

The therapeutic potential of human yCD::UPRT-AT-MSCs is universal and quite effective. This efficacy can be attributed to several factors, including the contribution of suicide gene vector and the tumor-tropic ability of the vehicle-mesenchymal/stromal cells. The choice of transgene influences the therapeutic efficacy. Yeast cytosine deaminase was shown to produce a 15-fold higher amount of 5-FU compared with bacterial cytosine deaminase [27]. Moreover, construction of the bifunctional fusion gene yCD::UPRT was reported to shortcut the rate-limiting enzymatic steps of the 5-FC/5-FU conversion, thus resulting in 10,000-fold sensitization of transgene-expressing cells to 5-FC [28].

Fusion yeast CD::UPRT gene-mediated prodrug therapy had significant antitumor effects in experimental animals. The yCD::UPRT-AT-MSCs suicide system was shown to be effective against 5-FU-resistant human primary cancer cells [29]. Our results support this finding by showing that C6 glioblastoma cells enriched for 5-FU resistant cells did not differ in sensitivity to ThSC-mediated killing in vitro [13]. The superiority of fused gene yCD::UPRT over the bacterial CD was previously demonstrated on two human glioma cell lines using an adenovirus vector gene therapy strategy [30,31].

The efficiency of 5-FC conversion by a bifunctional CDy::UPRT fusion gene led to both effective direct and bystander tumor cell killing in vivo. Uracil phosphoribosyltransferase (UPRT) is a pyrimidine salvage enzyme that catalyzes the synthesis of UMP from uracil and 5-phosphoribosyl-alpha-1-diphosphate. Formation of 5-FU by the yeast CD::UPRT fused gene causes inhibition of both DNA and RNA synthesis, consequently leading to the death of both dividing and nondividing cells, thereby attacking glioma stem cells [32].

The ThSCs described herein contribute significantly to therapeutic efficacy. AT-MSCs share some characteristics with pericytes [33,34]. The efficiency of MSCs-targeted therapy for glioblastoma may in part be explained by the pericycle-like properties of adipose-derived MSCs. It has been shown that synergistic targeting of both tumor endothelium and tumor pericytes affects tumor vascularization and tumor growth [35]. Injection of CDy-AT-MSCs may therefore target glioblastoma stem cells known to reside within a perivascular niche [36]. It was also reported that adipose-derived genetically modified MSCs have binding affinity for the vascular system [37]. Migration of yCD::UPRT-AT-MSCs to highly vascularized glioblastomas with subsequent binding to the vascular niche, where glioma cells reside might be one explanation for the observed glioblastoma curative effect [7,13]. In addition it was found that yCD::UPRT-AT-MSCs induced the expression of proapoptotic genes in tumor cells [12]. The combination of MSC-mediated prodrug gene therapy with the apoptotic ligand TRAIL or MSCs producing BMP4 might improve therapeutic outcomes [38]. Clinical trials could show whether similar outcomes can be expected in patients if ThSCs are applied after neurosurgical resection of primary tumors. The superiority of yCD::UPRT over bacterial cytosine deaminase is a promise for more efficient therapy. A very similar clinical study entitled "A Pilot Feasibility Study of Oral 5-Fluorocytosine and Genetically- Modified Neural Stem Cells Expressing E. coli Cytosine Deaminase for Treatment of Recurrent High Grade Gliomas" is ongoing [39].

To the high efficacy of the CDy-AT-MSCs/5-FC system contributes our recent finding that ThSC release exosomes able to invade tumor cells and kill them in presence of 5-FC. AT-MSCs-CDy-UPRT exosomes contain the suicide gene mRNA and protein–cytosine deaminase fused to uracil phosphoribosyl transferase. The exosomes inhibited growth of human tumor cell lines and human primary glioblastoma cells in a dose dependent manner in vitro (Figure 3). This observation opens new exciting possibilities for cancer gene therapy of human tumors. MSCs-CDy-UPRT cells are prepared by retrovirus infection therefore our recent finding that ThSC release exosomes able to invade tumor cells and kill them in presence of 5-FC. AT-MSCs-CDy-UPRT exosomes can be used as stable factory for genetically engineered exosomes with suicide gene and with drugs or genes tailored for more specific therapeutic usage [40]. Composition of exosomes released from MSCs might be purposely modified to create therapeutically interesting exosomes for cancer treatment [41]. Recently, it was published that if MSCs are treated with Paclitaxel, the drug is incorporated into exosomes [42]. Therapeutically active exosomes released from MSCs-CDy-UPRT cells supports early observation that MSCs driven prodrug gene therapy might the modality for treatment of pontine brainstem glioma the most malignant and dismal cancers in children [43].

Conclusions

Human mesenchymal stem/stromal cells expressing yeast cytosinedeaminase::uracil phosphoribosyl transferase migrate to tumors and convert the nontoxic prodrug 5-fluorocytosine directly

Figure 2: Rat glioblastoma C6 growth inhibition mediated by intracerebral application of various numbers of human CDy-UPRT-AT-MSCs. (Modified from Int. J. Cancer. 2012, 130: 2455-2463).
within the tumor mass to 5-fluorouracil, thus avoiding systemic toxicity. Prodrug administration not only eliminates tumor cells, but presence of suicide gene kills the genetically modified stem/stromal cells as well. The therapeutic potential of CDy-UPRT/5-FC suicide system mediated by stem/stromal cells is the most effective tumor targeting experimental therapeutic modality. Schematic illustration of MSC mediated prodrug gene therapy for cancer leading to its high therapeutic efficacy is depicted in Figure 4. Results support arguments for beginning clinical studies for the treatment of high grade tumors and metastases.

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