Translational Medicine

Extended Abstract

Translational Medicine 2015: Usage of engineered mesenchymal stem cells in contradiction of cancers - Isabelle De Waziers - Paris Descartes University

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Abstract

Gene-Directed Enzyme Pro-Drug Therapy (GDEPT) consists in expression of a suicide gene in tumor cells allowing in place conversion of the pro-drug into cytotoxic metabolites. During a previous work, we demonstrated that the mixture of a mutant CYP2B6 with NADPH Cytochrome P450 reductase (CYP2B6TM-RED), as a suicide gene, and Cyclophosphamide (CPA) might constitute a strong treatment for solid tumors (Touati et al, Curr Gene Ther, 2014). The efficiency of this mix was mainly due to i) an optimized suicide gene ready to metabolize efficiently CPA ii) an efficient bystander effect, iii) the event of an antitumor immune reaction. Major impairments were the directing of the suicide gene exactly to the tumor cells and its low appearance into tumor cells. Indeed, MSCs possess an unprecedented ability to home into tumors due to the inflammatory mediators which are found at the location of a tumor. MSCs are often easily isolated, from tissues like bone marrow (BM-MSCs) and fat (ACS), expanded in culture and efficiently transduced with recombinant viral vectors resulting in important and stable expression of the suicide gene. Once the transduction is performed, the foremost efficient clone for bioactivation of the prodrug are often selected and used for several patients. Indeed, given the minimal expression of the main histocompatibility complex MHC-I and MHC-II, allogeneic expanded Adipose-derived Stem Cells (eASC) delivered locally are well-tolerated and currently in clinical phase III clinical trial clinical trials for the treatment of inflammatory and auto-immune diseases (www.tigenix.com). MSCs expressing luciferase were wont to follow the longer term of MSCs after their intratumoral injections in animal models. Intratumoral injections of CYP2B6TM-RED-MSCs and CPA allowed an entire eradication of the tumor in 33% of the mice with none recurrence four months later. Different experiments are now under investigation to enhance the efficiency of this strategy.

Keywords: Mesenchymal stem cells, Gene-directed enzyme pro-drug therapy, suicide gene.

Introduction: The poor prognosis of patients with different cancers due to insufficient treatment efficacy indicates the need to explore simpler anti-tumor therapy. The broad application of agents that control cell proliferation is restricted by their short biological half-life or excessive toxicity. Recently, cell-based therapy has been considered a promising approach to reinforce anti-cancer effect. Among different cell types, mesenchymal stem cells (MSCs) have attracted increased attention, because they exhibit unique biological properties in vivo. Accumulating evidence indicates that MSCs transplanted in several pathological conditions are home to the sites of tissue injury and induce the recruitment of endogenous cells, tissue remodeling, and anti-inflammatory activities.

It has been recently shown that MSCs even have an aptitude to migrate toward tumors, being attracted by the plethora of chemo-attractants facilitating cell homing to active cancer sites with posterior trans differentiation due to the local micro environmental cues. The population of cancer-attracted MSCs actually supports the tumor growth and progression in several cancer types. However, anti-tumor properties of MSCs have also been reported, rendering them very attractive to researchers and clinicians. To bypass the matter with the duality of MSC influence on the tumor cells, a delivery of exogenous, engineered MSCs could present some solution for converting them into the unequivocal therapeutic tools.

The engineering strategies of MSCs equip them for targeted delivery of various factors using more focused biological approaches. MSCs are often modified to become the carriers of suicide genes, which, in turn, would produce toxic products that might inhibit tumor expansion, whereas the encompassing healthy tissues remain intact. MSCs can also be used because the carriers of anti-angiogenesis factors that contributes to the inhibition of tumor growth and to stop metastasis. Yet one more approach is that the induction of cytokine organic phenomenon in MSCs, which, in turn, will attract and modulate processes, making the tumor cells more exposed to the host system response. Besides this, anti-mitotic factors might be a rational target for the MSC-based anti-

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cancer engineering.

Ultimately, rising interest is focused on the operation of exosomes as biological delivery vehicles for miRNA transfer, as exosomes don't elicit acute immune refusal and risk of tumor creation.

In this article, we'll specialize in some recent advances in cell-based cancer therapies using genetically engineered MSCs also as on the potential side effects of MSC delivery strategies.

A. Heterogeneity of MSCs: In the 1970s, Friedenstein and his coworkers identified within the bone marrow a subpopulation of nonhematopoietic cells with a fibroblast-like morphology designated as colony-forming unit fibroblasts. Afterward, the term "MSCs" was adopted by the Caplan group to define a population of stem cells with a three-lineage differentiation potential.

In 2006, the International Society for Cell Therapy (ISCT) proposed the minimal criteria for MSCs: adherence to plastic when cultured in vitro; possession of a trilineage mesodermal differentiation capacity toward chondrocytes, osteocytes, and adipocytes. Additional requirements for MSCs include the expression of the cell surface molecules like CD73 (ecto 5′ nucleotidase), CD90 (Thy-1), and CD105 (endoglin) also because the absence of hematopoietic markers, including CD45, CD34, CD14 or CD11b, CD79α, and therefore the MHC II class cellular receptor HLA-DR.

However, these criteria are proved to be inadequate. The expression of this broad set of markers was also found on fibroblasts and on the surface of the opposite cell types. In fact, the isolation of MSCs reliable with ISCT criteria produces heterogeneous, committed progenitors, nonclonal cultures of stromal cells containing stem cells with different multipotential properties, and differentiated cells. additionally , it had been recently postulated that only a minor subpopulation of pluripotent stem cells among MSCs, called multilineage-differentiating stress-enduring (Muse) cells, are liable for the broad spectrum of differentiation abilities previously attributed to the entire MSC population.

Moreover, despite the similar pattern of surface antigen expression, global expression patterns vary significantly in MSC population isolated from bone marrow, fat, and duct blood. Numerous publications indicate that MSCs have multiple developmental origins and belong to pericytes, fibroblasts, or neural crest cells but issues regarding ontogeny of MSCs are still very controversial. Clonal assays demonstrated that in an MSC population, multiple sorts of cells with different developmental potential exist.

Due to this heterogeneous nature of MSCs, their precise characterization within the absence of known accurately defining biomarkers poses a challenge for his or her further use in cell therapy. However, the issues with detailed identification shouldn't interfere with future investigation of their therapeutic properties, albeit the results among studies somewhat vary.

B. Inherent Anti-Cancer Properties of MSCs: Several studies postulated that naïve, non-engineered MSCs may exert anti-tumor activities. However, it should be noted that this alluring from the therapeutic point-of-view feature of MSCs remains under debate due to contradictory data reported on MSC influence on tumor cells. As an example, MSCs derived from different tissues may stimulate or suppress glioblastoma cell proliferation as reported by Akimoto et al. that adipose tissue–derived MSCs (AT-MSCs) induced and duct blood–derived MSCs (UCB-MSCs) inhibited the progression of the glioblastoma cells.

Even more remarkably, MSCs of an equivalent origin, cultured within the same conditions in vitro, promote or restrain tumor progression counting on the protocol applied during the experiment, that is, human Fallopian tube MSCs (hftMSCs) utilized in a murine breast adenocarcinoma study. During this study, hftMSCs participated in tumor progression when coinjected subcutaneously with tumor cells, but they exerted antitumor effects when administrated intraperitoneally to the animals already bearing tumor cells.

Nonetheless, to limit the story to the productive side of MSCs, there are several samples of beneficial effects elicited by the unmodified MSC administration in various cancer types, suggesting that MSCs do possess intrinsic anti-tumor properties that are deserve interest and further investigation. There are several prominent examples from anti-glioblastoma experiments, followed by other anti-cancer MSC application like in carcinoma, cancer of the liver, carcinoma, prostatic adenocarcinoma, carcinoma, myeloma, and sarcoma, up to the case of the human BM-MSCs use in an anti-lymphoma. Interestingly, neither positive nor negative effects were attributed to human Wharton's jelly-derived MSC secretome on carcinoma cells in vitro. Due to their inherent ability to migrate toward lesion sites, MSCs seem to be very attractive in future anti-tumor therapies. As an example, UC-MSCs contributed to the increased overall anti-tumor effects once they were administrated in vivo into tumor-bearing mice followed by the therapeutic irradiation exposure. During this case, human tumor cells had been implanted to the dorsal skin

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folds to get bilateral xenotumors followed by the intraperitoneal MSC administration. Within the next step, just one tumor-containing site was irradiated, leaving the other tumor site for the study of "bystander effect". Due to their positive impact on radiotherapy effects, UC-MSCs might find their application within the tumor radio-sensitization approaches.

After BM-MSC administration into mice bearing hepatoma (HCC), up regulation of p53 and caspase-3 genes was reported in liver tissue, which eventually led to the apoptosis induction. Additionally, tumor cells were found to be within the majority in the G0/G1 phase, with the concomitant S-phase decline.

In vivo, UCB-MSCs triggered PTEN stimulation not only in glioblastoma cells that were in direct contact but also in those within the vicinity. PTEN upregulation influenced Akt expression pattern, causing PI3K/Akt pathway disruption and resulting in the inhibition of growth and migration of cancer cells.

Glioblastoma tumor progression needs new vessel formation. Ho et al. indicated that BM-MSCs could exert anti-tumor effects during this regard. It had been shown that glioma tumor angiogenesis could be unsettled by BM-MSC paracrine action on endothelial progenitor cell recruitment, with concomitant downregulation of proangiogenic factors. Further, BM-MSC secreted factors that inhibited endothelial cell tube formation in-vitro and took part within the decline of microvessel density in vivo within the subcutaneous glioma tumor mouse model.

In an in-vitro study, glioblastoma multiforme stem-like cells entered senescence, but not apoptosis, with an indicator of cyclin D1 increased level on account of exposure to BM-MSC conditioned media. An increased sensitivity to the anti-cancer drugs, that is, Temozolomide and 5-Fluoro-Uracil, was also noticed. Similar results were observed within the case of human carcinoma cells that were co-cultured with AT-MSCs.

Another in-vitro study brings data on apoptosis and differentiation induction within the U251 human glioma cell line after stimulation with MSC conditioned media from different MSC origins: AT-MSCs and UC-MSCs. during this study, after the MSC conditioned media exposure, elevated levels of mRNAs for caspase-3 and -9 were detected with the simultaneous decline in mRNA levels of survivin and X-linked inhibitor of apoptosis protein (XIAP) in U251 cells. Additionally, G0/G1 growth cell arrest had been reported in these cells. Besides, MSC conditioned media called out U251 cell differentiation toward normal phenotype glial cells that were manifested with GFAP presence and cell migration decline. On the opposite hand, there are some data claiming that MSC origin is crucial, because different tissue-derived MSCs act in completely alternative ways.

Culture medium from osteo-induced AT-MSCs contributed to the in vitro proliferation inhibition and apoptosis induction in carcinoma cells. Such a positive influence could be dependent not only on the differentiation state of AT-MSCs but also on the origin of the therapeutic MSCs. These data are reinforced by observation of the immortalized MSC line that produced and secreted tissue inhibitor of metalloproteinase-1 and -2 (TIMP-1, TIMP-2). The elevated presence of TIMP-1 and TIMP-2 within the extracellular matrix, undoubtedly, interferes with the tumor cell migration processes that are crucial for tumor progression through metastasis.

C. Suicide Gene Methods: The herpes simplex virus-thymidine kinase (HSV-TK) became one among the foremost used enzymes employed in suicide gene anti-cancer approaches. Within the case of MSCs, the advantage of this approach is that HSV-TK modified MSCs might be effectively delivered to the world of interest and ganciclovir, a substrate for HSV-TK, might be safely administrated systemically. The toxic product is exclusively produced from ganciclovir by the HSV-TK-bearing MSCs, which, in practice, would target the region occupied by tumor cells. As a consequence, only tumor cells are going to be affected, whereas surrounding tissues would remain intact.

HSV-TK engineered MSCs act mainly through the "bystander effect," which consists of an action of the toxic compound not only within the cells where it had been produced but also after its delivery to the encompassing target cells. Additionally, this mechanism is reported to be very effective for anti-tumor acting BM-MSCs. Within the case of HSV-TK-based anti-cancer cell therapy, the gap-junctional intercellular communication system between therapeutic and target cells is crucial due to its involvement within the toxic compound transmission; thus in practice, this phenomenon may cause the insufficient toxic compound penetration throughout the tumor site, resulting in incomplete treatment. For this reason, advanced monitoring methods are desired to exert control over ongoing processes in vivo.

Finally, the inducible Caspase-9 (iCas-9) suicide gene system might find its application within the MSC-based anti-tumor therapies. The iCas-9 system includes activation of the Caspase-9 by the small-molecule chemical inducer of dimerization (CID), which is meant to interact with the iCasp9. As an example, iCas-9-producing BM-MSCs treated

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with CID initiated apoptotic pathways, causing their relatively fast clearance in vivo. This feature was utilized within the anti-lung cancer BM-MSC-based experiment. During this case, the Bortezomid stimulation of the iCas-9-producing BM-MSCs led to the rise of pro-apoptotic activity via the Caspase-3 stabilization, confirming feasibility of the iCas-9 system usage within the field of anti-tumor activities.

D. Tumor Propagation Mediated by MSCs: Some features of MSCs like immunomodulation or paracrine effects, which can be beneficial in regenerative medicine approaches, might be also destructive in cell therapy against cancer. Some studies have shown MSC contribution in tumor growth, metastasis, and development of anti-cancer drug resistance.

Results from co-culture of MSCs, carcinoma cells, and peripheral blood mononuclear cells (PBMCs) showed that MSCs negatively affected proliferation and migration of PBMCs and favored the shift from Th1 toward Th2 response. A rise within the number of regulatory T cells (Tregs) was also observed, which might be a minimum of partially due to MSC-derived anti-inflammatory cytokine, TGF-β. In vivo studies on mammary carcinoma-bearing mice showed that intravenously administered human peripheral blood–derived MSCs (human PB-MSCs) might support tumor growth through stimulating the Tregs and secretion of immunosuppressive factors, that is, TGF-β, IL-4, and IL-10 with a simultaneous decrease within the cytotoxic capacity of CD8 T lymphocytes and NK cells.

Furthermore, MSCs infiltrate tumors and support their progression. It had been observed that the bulk of the mouse glioma cell line (GL261) consisted of cells with mesenchymal features (Lin-Sca-1+CD9+CD44+CD166+ phenotype; trilineage differentiation capacity). However, most of the MSC-like cells during a tumor mass were of host origin, which indicated that endogenous MSCs were recruited to the tumor site. Moreover, brain tumor–derived MSCs co-injected with GL261 cells significantly reduced mice survival rate, compared with GL261 cell injections alone. Similarly, other studies on different tumors like head and neck cancer, colorectal cancer, or carcinoma also confirmed the positive effect of MSCs on tumor growth.

Similarly, doxorubicin resistance was observed among triple-negative carcinoma cells after AT-MSC conditioned medium treatment. Further studies revealed that the medium containing IL-8 caused a rise in BCRP protein carcinoma resistance protein, one among the ABC transporters that are ATP-binding cassette transmembrane proteins engaged within the transport of a good range of molecules across cell membranes, frequently against their concentration gradient, resulting in the multidrug resistance just in case of cancer cells).

Other results showed that MSC-derived exosomes could restrain apoptosis of gastric cancer cells and enhanced the expression of multi-drug resistance-associated proteins after exposure to 5-fluorouracil. In turn, Vianello et al. reported that co-culturing chronic chronic myelocytic leukemia cells with BM-MSCs prevented them from imatinib-induced apoptosis via the SDF-1/CXCR4 axis. Inhibition of CXCR4 abolished this resistance.

Because of the heterogeneity of the MSC population, Waterman et al. proposed a distinction between those cells into two phenotypes. Consistent with this hypothesis, MSCs are often polarized, counting on which Toll-like receptor (TLR) was previously activated on the cell surface, into pro-inflammatory MSC1 (TLR-4-primed) or immunosuppressive MSC2 (TLR-3-primed). Both in vitro and in vivo studies showed that MSC1 hampered tumor cell growth and invasion, whereas MSC2 supported these processes.

Conclusion: Genetically modified MSCs seem to be a promising strategy to enhance cell-based therapy, enabling delivery of a plethora of things that effectively repress tumor growth. A review of currently published studies has shown that the consequences of MSCs engineered to precise different genes or to function a vehicle for therapeutic agents for cancer therapy are multiple and should depend upon the state of tumor and interactions with other cell types within the tumor microenvironment. These results are sometimes contradictory, and therefore the factors released from modified MSCs are implicated in both pro- and anti-tumor growth and/or metastases. Hence, the selective control of therapeutic organic phenomenon by MSCs within the defined tumor settings provides new options to use modified MSCs for cancer therapy in patients.

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