

REVIEW

Genetically engineered mesenchymal stromal cells in cancer gene therapy

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ABSTRACT

Based on our experimental data, we aimed to emphasise the perspectives of the use of mesenchymal stromal cells (MSC) in the cancer gene therapy. On the other hand, we would like to point out factors which should be taken into consideration at their clinical use. In this review we define MSC as unique targets for targeted therapy. We proved the efficacy of experimental therapeutic approach utilising enzymatic conversion of non-toxic prodrug into chemotherapeutic by engineered MSC, and we observed significant cytotoxic effect in many preclinical models including metastatic disease. Treatment was enabled by affinity of MSC to tumour tissue and subsequent delivery of therapeutic molecule into the tumour. We also observed decreased efficacy of cell-mediated gene therapy on chemoresistant tumour cells. Moreover MSC can exert a supportive effect on tumour cells as well as to decrease the efficacy of conventional treatment. Besides obvious unique benefits connected to the use of MSC we pointed also to possible risks associated with their clinical application (Ref. 24). Text in PDF www.elis.sk. KEY WORDS: mesenchymal stromal cells, cancer gene therapy, prodrug-converting genes, chemoresistance, tumour microenvironment.

Introduction

Despite advances in diagnostics and therapy, cancer belongs to the major causes of death in western countries. Conventional treatment is accompanied by adverse effects with negative impact on patients' quality of life. Chemoresistance and metastatic dissemination of tumour cells mostly lead to death of oncological patients. It is necessary to search for innovative therapeutic approaches. Cancer gene therapy utilizing enzymatic conversion of non-toxic prodrug into active cytotoxic compound has been used as experimental treatment for many years. Viral vectors, different types on nanocarriers or physical methods have been used for delivery of therapeutic gene into target cells. Preclinical research brought promising results, but clinical studies failed because of inefficient transfer of genetic information into target cells, insufficient infiltration of tumour tissue by vector or low expression of therapeutic gene, respectively. The use of MSC as vehicles for therapeutic genes has enabled to overcome these obstacles as rev in (1).

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Acknowledgement: Slovak Research and Development Agency contracts APVV-0052-12 and APVV-0230-11, RFL and WAC programs funded by the Slovak Cancer Research Foundation, the League against Cancer, VEGA grants No. 2/0171/13 and 2/0130/13.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSC) represent a population of non-hematopoietic multipotent cells of fibroblastoid morphology. Originally they were identified in bone marrow (2). Till now they have been found almost in all organs (3) located on the abluminal side of blood vessels (4). To the most often used sources of MSC in research as well as in clinical practice belong bone marrow, adipose tissue, dental pulp, umbilical cord or umbilical blood, respectively. The natural function of MSC is to support homeostasis; they contribute to regeneration and wound healing. Based on paracrine intercellular signalling they enter blood stream and home in the side of injury or inflammation (5). The International Society for Cellular Therapy approved a consensus of minimal criteria characterising MSC: *ex vivo* they are defined as plastic-adherent cells when cultured in standard conditions, and they are able to differentiate into osteogenic, adipogenic and chondrogenic lineages. More than 95% of the cells must express surface markers CD73, CD90 and CD105, and they must lack expression of CD45, CD34, CD14 or CD11b, CD79 α or CD19 a HLA-DR (6). There is an effort to search for new criteria based on advanced methods such as analysis of transcriptome, proteome and secretome which would enable more accurate definition of MSC (7), (8) (1).

Gene therapy mediated by mesenchymal stromal cells

The finding that MSC possess along other unique features also high affinity to tumour tissue enabled their usage as targeted delivery vehicles for therapeutic molecules, which significantly

contributed to progress in cancer gene therapy. Genetically engineered MSC deliver the therapeutic gene to the tumour based on paracrine signals released by tumour imitating injured tissue (9). These signals are recognised by MSC, they home in tumour and subsequently they become an integral part of tumour stroma. The use of cellular vehicles in gene therapy significantly reduced limitations related to viral or non-viral vectors.

In our laboratory we have been using adipose tissue-derived MSC (AT-MSC) for this purpose. Adipose tissue is really convenient source of MSC; it can be obtained in sufficient quantity from healthy donors at liposuction or plastic surgery. The next advantage is relatively big proportion of AT-MSC within nucleated cells in adipose tissue. The frequency among nucleated cells in bone marrow is 1:50,000 – 1:100,000, the frequency of MSC in adipose tissue is 500-fold higher (10).

After isolation of AT-MSC by collagenase digestion and plastic adherence we used retroviral vectors for genetic modification. Retroviruses integrate the therapeutic gene into genome of the host cell, and they provide stable expression of transgene and vertical transfer into daughter cells.

More approaches can be used in cancer gene therapy. It is possible to suppress the expression of activated oncogenes, restore the expression of tumour-suppressor genes, inhibit tumour angiogenesis and metastatic potential of tumour cells or activate anti-cancer immunity. Prodrug-converting genes represent important group of therapeutic genes used for gene-directed enzyme prodrug therapy (GDEPT). They encode enzymes which convert non-toxic prodrug into toxic product (11). Our group has been focusing on this type of gene therapy. For genetic modification of AT-MSC we use genes which do not have their equivalent in mammalian cells – ‘normal’ mammal cells are not able to activate the prodrug. Only engineered cells are capable to convert prodrug to active chemotherapeutic. This approach has been called ‘suicide gene therapy’ since it causes the death of transgene-expressing cells. For cell-mediated approaches it is not the primary aim to kill the engineered cell (although this phenomenon increases the safety of this therapeutic approach). The cell-mediated approach is based on bystander effect – the aim is to induce apoptosis in neighbouring tumour cells by release of drug converted by therapeutic cells.

The main advantage when compared to conventional chemotherapy is cytotoxic effect localised to the tumour, where prodrug and activating enzyme get together.

We demonstrated that therapeutic systems using fusion gene yeast cytosine deaminase:uracil phosphoribosyl transferase (CD::UPRT) with prodrug 5-fluorocytosine (5-FC) or *Herpes simplex virus* thymidine kinase (HSVtk) with ganciclovir (GCV) exert high efficacy on many types of tumour cells *in vitro* as well as on mice models. After systemic administration of therapeutic cells we observed significant inhibition of growth of subcutaneous xenotransplants derived from colorectal cancer (12) or melanoma (13). In this melanoma model we also demonstrated that more than 80 % of treated mice survive for long-term without relapse (14).

We also achieved relevant results on orthotopic glioblastoma multiforme (GBM) model. No effective treatment exists for this aggressive malignant disease, and the prognosis for patients suf-

fering from GBM is very unfavourable. We demonstrated that genetically engineered AT-MSC maintain the tumour tropism when they are intracranially administered into distant regions from xenotransplant induced by rat GBM-derived cells C6. The use of osmotic pumps enabling continuous administration of 5-FC and repeated injection of therapeutic cells even prolonged the survival of experimental animals (15). Study which simulated the therapeutic regimen of patients suffering from GBM revealed that CD:UPRT-MSC/5-FC treatment possess curative potential leading to long-term survival of rats, which underwent surgical resection of tumour and subsequent administration of therapeutic cells and continuous administration of 5-FC (16).

We have also focused on treatment of metastatic disease. Ovarian cancer belongs to diseases with unfavourable prognosis because of chemoresistance and abdominal metastases. We achieved long-term survival by CD:UPRT-MSC/5-FC treatment in one third of metastases-bearing mice (17). In a model of lung metastases induced by breast cancer-derived cells we proved that systems CD:UPRT-MSC/5-FC and HSVtk-MSC/GCV act in synergic manner. By combination of two mentioned approaches it is possible to eliminate tumour cells which are resistant to particular systems (18).

On the other hand we demonstrated that intrinsic properties of tumour cells play a key role in efficacy of cell-mediated cancer gene therapy. The ability of gap-junctional intercellular communication (GJIC) is critical especially for HSVtk-MSC/GCV system, because phosphorylated GCV is not able to diffuse across cell membranes, and it passes from therapeutic to target cells via connexin intercellular junctions (19). We observed low efficacy of HSVtk-MSC/GCV approach on tumour cell lines deficient in GJIC. The expression level of enzymes involved in nucleotide metabolism is very important for treatment efficacy as well as expression of ABC transporters which are responsible for efflux of chemotherapeutics from tumour cells into extracellular space (20).

We demonstrated that various types of tumour cells differ in ability to stimulate the homing of MSC into tumour. Despite promising results *in vitro*, in mice model we did not achieve significant inhibition of subcutaneous xenografts induced by glioblastoma-derived cell line 8-MG-BA. We assume that insufficient infiltration of tumour by therapeutic MSC caused the failure of therapy (19).

A pilot study with genetically-modified neural stem cells expressing *E. coli* cytosine deaminase for treatment of recurrent high grade gliomas was completed (21), and the second similar study has been active in United States (22). The first European study TREAT-ME1 aimed to assess the safety and efficacy of therapy by autologous MSC isolated from bone marrow transduced with retroviral vector coding HSVtk in combination with GCV in advanced gastrointestinal cancer started in 2015 (23).

A study defining the efficacy of gene therapy mediated by cellular vehicles on chemoresistant populations of tumour cells or cancer stem cells has not been published yet. Chemoresistance of tumour cells is a phenomenon which occurs very often, and it complicates or even disables the cancer treatment. Therefore we focused also on evaluation of efficacy of this therapeutic approach on chemoresistant cells. We cultured tumour cells derived from colorectal carcinoma in consecutively increasing concentration of

5-FU, and we prepared chemoresistant derivative HT-29/EGFP/FUR, which proliferate in plasma-relevant concentration of 5-FU. The resistance as well as expression of genes associated with aggressive phenotype increased with numbers of passages, and it negatively influenced the efficacy of cell-mediated cancer gene therapy. We pointed out that it is important to consider previous therapy and possible resistance when defining inclusion criteria for patients entering clinical studies (Durinikova et al., submitted).

In clinical use of MSC it is important to take into consideration that MSC (or cells derived from them) contribute to tumour microenvironment, where they influence the properties of tumour cells. As mentioned above, MSC expressing prodrug-converting genes are affected by toxic metabolites produced by inserted enzyme and despite their relative high resistance they undergo apoptosis in time. This fact enables GDEPT mediated by MSC to be declared safe.

Non-engineered MSC are used in regenerative medicine, plastic surgery, for treatment of autoimmune diseases and GVHD. It was proved that MSC as integral part of tumour microenvironment can support proliferation, invasiveness, and they can decrease the sensitivity to chemotherapy. We demonstrated that MSC cultured in the presence of cisplatin differ from cells cultured without chemotherapeutic. Alterations in secretion and phosphorylation profile of MSC subsequently negatively influenced the response of breast cancer-derived cells to chemotherapy, and supported expression of cancer stem cells' markers (24). It is necessary to understand the tumour cells – MSC interplay to maximise the safety of cell therapies.

Learning points

The therapeutic potential of MSC is incontestable. Several clinical studies using MSC or progenitor cells for treatment of various conditions have been conducted. MSC are used in the treatment of autoimmune disorders and graft-versus-host disease (GVHD), as well as in regenerative medicine and plastic surgery. These unique cells enabled significant progress in cancer gene therapy. On the other hand it is necessary to think of the fact that MSC can exert supportive effect on tumour cells, and they can decrease the efficacy of conventional cancer treatment.

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Received December 21, 2017.

Accepted January 9, 2018.