

Antitumoral cell-based therapies

Review Article

Javier García-Castro*, Daniel Rubio, Ricardo de la Fuente, Antonio Bernad

Department of Immunology and Oncology, Centro Nacional de Biotecnología (CSIC), Madrid, Spain

*Correspondence: Javier García-Castro; Phone: +34 915854656, Fax: +34 913720493, e-mail: jgarcia@cnb.uam.es

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Summary

Cell therapies are based on biological agents involving cells to be administered to patients with diverse diseases. Examples of cell-based therapies include implantation of cells as an *in vivo* source of an enzyme, cytokine or factor; infusion of immune cells such as lymphocytes, or transplant of cell populations such as hematopoietic cells, hepatocytes or pancreatic islet cells to perform a complex biological function. A similar concept can be applied to cancer in a new antitumor approach. In this case, carrier cells are usually modified *ex vivo* by vectors or by pre-loading with bioactive materials such as toxins or viruses. Several cell types target naturally to the tumor mass, or are engineered to improve this preferential homing.

I. Introduction

Current cancer treatments are based on systemic drug administration. It is often difficult to obtain high intratumor concentration of these agents because of unacceptable secondary effects. Significant advances have been made in the development of new therapies with specific tumor targeting, as is the use of antibodies or viral vectors (Viti et al, 2002; Galanis et al, 2001). These agents nonetheless do not home specifically to the tumor and are affected by problems including limited half-life in the bloodstream, non-specific adhesion, as well as difficulty in extravasation and immune response. An ideal candidate would thus be a carrier with properties for specific tumor targeting, capacity for extravasation, which does not present problems to the immune system.

Tumors may comprise different cell types: fibroblasts, stroma, immune cells, endothelial progenitors, and the heterogeneous cancer cells themselves (Figure 1). Since cancer cells may produce a variety of factors, a tumor can recruit certain cells (e.g., lymphocytes, macrophages or endothelial progenitors) during its development. During the hyperproliferative stage, tumors induce surrounding tissue to support new blood vessel formation, mainly through production of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) (Rafii et al, 2002). Other factors secreted by malignant cells, such as stromal-derived factor-1 (SDF-1), induce migration of certain immune system cells to the tumor. This migration can be produced indirectly, since

SDF-1 can induce integrin upregulation, aiding adhesion and ligand-dependent transmigration of vascular endothelial cells (Zou et al, 2001).

In contrast to systemic drugs currently in use, cells do not distribute randomly via the circulation, but have an intrinsic program for trafficking through the body and entry into organs (Figure 2). A heterogeneous cell population in solid tumors resides in a common stromal microenvironment that is defined by interaction of this population with neighboring cells and local factors such as cytokines, molecules and extracellular matrix (Mareel and Leroy, 2003). Little is known of the interactions among these components that support tumor growth or are involved in tumor rejection (Stetler-Stevenson et al, 1993). Despite this lack of knowledge, it is possible to use cells as carriers to induce tumor inhibition. If a cell could be loaded with antitumor agents, a more specific cell-based therapeutic strategy would be possible, inducing powerful local action on the tumor. Here we will summarize some of the recent progress using cells as carriers of therapeutic products, focusing mainly on review of recent clinical trials.

II. Cellular vehicles

Cells of the immune system including T cells, macrophages, NK cells and eosinophils or cells related to tumor neoangiogenesis are the most obvious choices as vehicles, but other cell types such as tumor or stem cells could also be used.

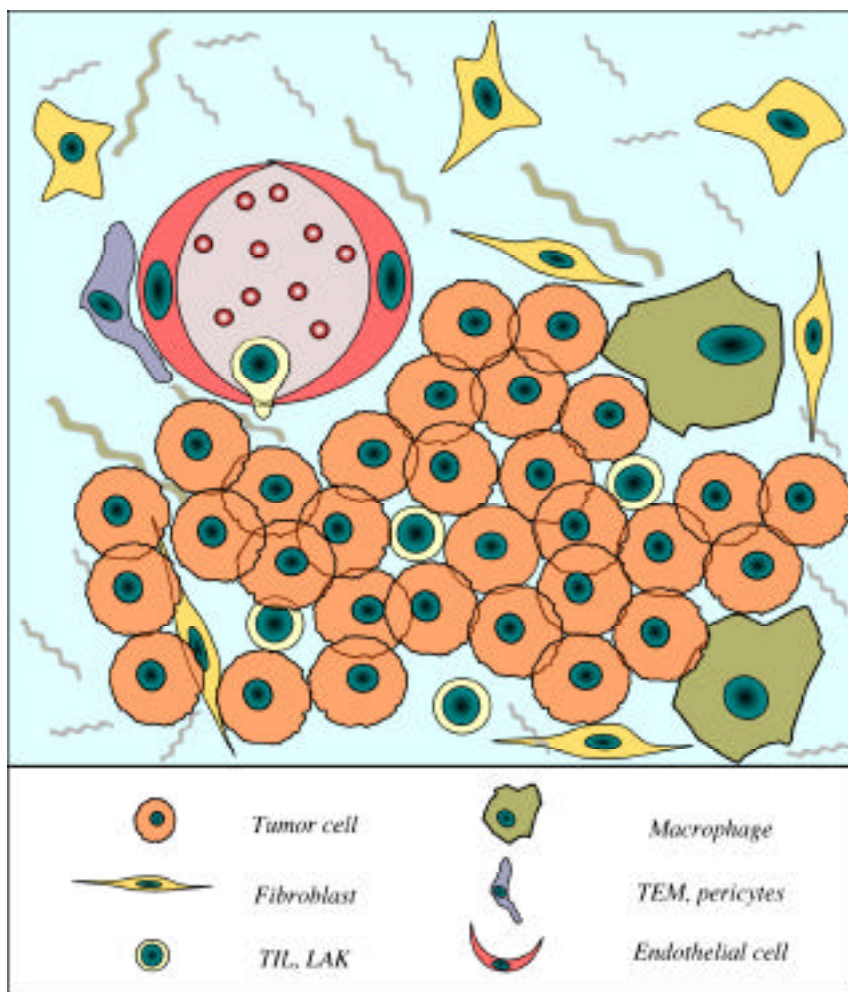


Figure 1. Cellular diversity in the tumoral microenvironment. Schematic overview of different cellular types related to the progression of cancer cells. Many of these cells could be used as cellular vehicles in antitumoral therapies in base to their properties related with a specific recruitment to tumor sites.

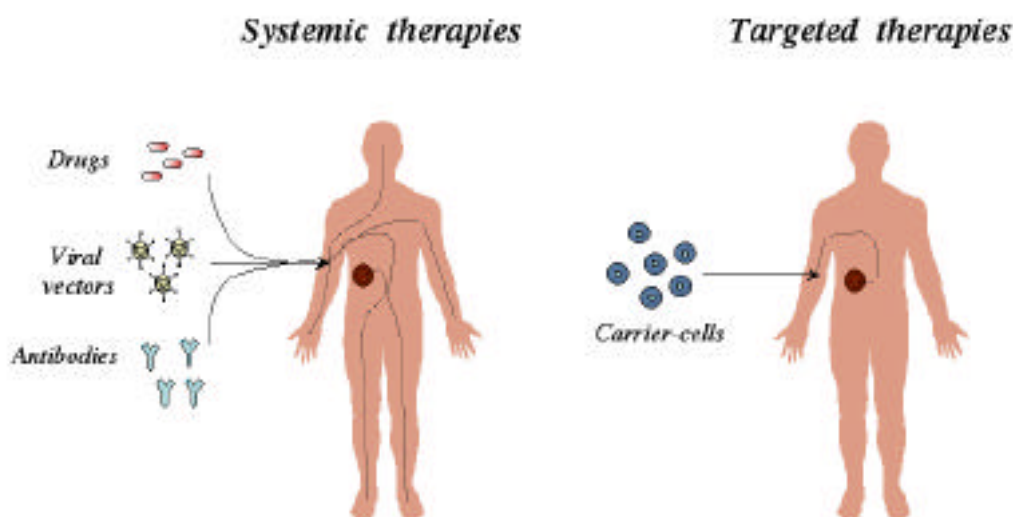


Figure 2. Physiological models of distribution through the body of the therapeutic agents. In “systemic therapies”, classic drugs or new therapeutics, as viral vectors or antibodies, are administered to the patient and quickly they are distributed by all the body. They reach a homogenous concentration, which can have therapeutic effects but also, sometimes, undesirable secondary effects. By contrast, with “targeted therapies”, using carrier cells loaded with antitumoral agents, we could deliver therapeutics without problems of systemic dilution, minimizing collateral damage and with highly locoregional concentration in base to natural properties of tumor-homing cells.

The ideal features of a candidate are a) immunological silence, b) a large number of easily-obtained cells, c) susceptibility to vector transduction (**Table 1**), d) lack of non-specific adhesion in the bloodstream, e) specific extravasation and homing to the tumor site. No perfect cell vehicle is currently available, although many approaches have been tested.

A. Immune cells

Despite the theoretical suitability of immune system cells as vehicles to transport therapeutic products to the heart of the tumor, most studies using these cells center mainly on activating their innate immune capacity. Activation of the immune system is nonetheless a complex process. It requires not only immune cell localization to the tumor, but also an effective immune cell:tumor cell ratio, and adequate signaling through the TCR/CD3 complex plus a co-stimulatory signal (via ligands of the B7 family). In addition, the effect of suppressor cytokines must be avoided, since cytokines such as TGF- and IL-10 can be secreted by the tumor cell, by surrounding stromal cells, or even by the activated immune cells themselves. The results of clinical studies in immunotherapy will be outlined below, although additional work will be required to determine the validity of immune cells as bystander therapeutic carriers.

1. NK and T cells

Tumor immunotherapy is a growing field, thanks to recent descriptions of factors implicated in the immune response to tumors and tumor-associated antigens, and reports on the need for lymphocyte activation by dendritic cells. Nevertheless, tumor cells often fail to induce a specific immune response due to the lack of a tumor-associated antigen, lack of a costimulatory signal, or by producing immunological inhibitors (Song, 1998). Absence of adhesion receptors on tumor vessels may also prevent lymphocyte infiltration and contact with tumor cells (Oppenheimer-Marks et al, 1990). In spite of this, experiments in mice showed that T cells can inhibit tumor growth, although few clinical studies have been conducted and the clinical benefits of such treatments have not been clearly documented (Greten and Jafee, 1999).

Peripheral blood leukocytes can be cultured *in vitro* in the presence of several cytokines, particularly IL-2, to obtain lymphokine-activated killer (LAK) cells (Melder et al, 1989). Although some tissue-resident lymphocytes may have spontaneous LAK activity, normal blood mononuclear cells show no LAK activity, which is acquired only after incubation with IL-2 (Phillips et al, 1987). LAK cells have a wide spectrum of lytic activity against tumor cells in both autologous and allogeneic settings, whereas normal tissues are resistant to LAK-mediated lysis (Fox and Rosenberg, 1989). Combined infusion of LAK cells and IL-2 has been evaluated in clinical trials, in which antitumor effects correlated with IL-2 dose and the number of LAK cells administered (Yano et al, 1999). Certain aspects remain to be modified, such as use of continuous IL-2 infusion rather than bolus

injection to reduce systemic toxicity, or the need to select patients with low tumor burden and a large number of harvested NK cells. Nonetheless, results of clinical trials using LAK cells have shown efficacy in treatment of micrometastases, although not in large tumors (Kimura and Yamaguchi, 1997).

Tumor-infiltrating lymphocytes (TIL) are T cells with unique tumor activity, which infiltrate some tumors and can be expanded *ex vivo* with IL-2 (Mukai et al, 1999). Although data on the physiological behavior of TIL are limited, these cells have already been used clinically in antitumor therapies, especially in melanomas (Rosenberg et al, 1994). TILs were recovered from patient tumors, cultured *ex vivo* with IL-2, selected and expanded based on their tumor-specific reactivity, then reinfused to the patient. Nonetheless, most common "tumor-specific" antigens are in fact specific for the tissue or cell types that compose it; these antigens can also be expressed by normal tissues or cells of the same type (Song, 1998).

Table 1. Vectors for gene and cell therapy. Efficient gene transfer requires the use of a vector and, depending upon the strategy, advantages and disadvantages of each one have to be considered.

Type	Vector	Expression	Characteristics
Non-viral	Liposomes	Transitory	Low transfection efficiency. Good safety profile.
	Naked DNA or RNA	Transitory	Low transfection efficiency. Simple and cheap production.
	Molecular conjugates	Transitory	Flexible design. Unstable <i>in vivo</i> .
Viral	Retrovirus		
	Oncovirus	Prolonged	Integrated in proliferating cells.
	Lentivirus	Prolonged	Integrated in proliferating and non-proliferating cells.
	Adenovirus	Transitory	Very high transfection efficiency. No integrating. Generates immune response.
	Poxvirus (vaccinia)	Transitory	Great clinical experience.
	Adeno-associated virus (AAV)	Prolonged	Insert-size limit of 4.5 Kb.
	Herpes simplex virus	Transitory	Very efficient <i>in vivo</i> .

The activity of the TILs used in this protocol, selected for their ability to recognize tumor-associated antigens, may thus be less specific than desired.

A major obstacle in TIL-based therapies is that they can be cultured only from 50% of patients and several weeks of culture are required. Various authors nonetheless reported partial clinical responses in patients treated with TIL infusions, although their data also indicate that tumor size and the number of antigenic targets are essential for the success of TIL-based immunotherapy (Lister et al, 1995; Basse et al, 2000; deMagalhaes-Silverman et al, 2000). In these clinical trials, TIL localization within tumors was demonstrated after infusion, although there is also TIL homing to other organs such as liver and spleen, with potential autoreactivity. Moreover, when peripheral blood lymphocytes (PBL) were infused in patients as a control, no preferential trafficking pattern to the tumor was found for TIL versus PBL (Economou et al, 1996). All together, these data place the central hypothesis of preferential TIL homing to the tumor in doubt.

NK cells can also lyse tumor cells, and have the advantage that they are easily obtained from patient peripheral blood (Whiteside et al, 1998). Following systemic injection, these cells were found in large numbers, primarily in the tumor and in lung (Melder et al, 2001). NK cells do not require a second costimulatory signal for complete activation (Hombach et al, 1993), an additional advantage for immunotherapy. In contrast to TIL, however, there is no evidence of benefits in clinical trials using NK cells, although accumulation was demonstrated within tumor metastases (deMagalhaes-Silverman et al, 2000). These clinical protocols are based on the innate capacity of T and NK cells to home to the tumor and trigger an immune response. Several groups have focused on strategies based on enhancement of T cell cytotoxicity through gene transfer (van de Winkel et al, 1997; Weiner et al, 1997). Two main strategies are being tested to increase cytotoxicity in T cells. One is based on screening sequences for improved peptide-MHC binding and/or to increase the affinity of this complex with the TCR (Brockner et al, 1996; Chung et al, 1994). A second strategy focuses on activation of costimulatory signals, based on B7 and TNF family molecules (Hurwitz et al, 2000).

Different lymphocyte subsets vary in their ability to extravasate and reach sites of tumor growth. This capacity is dictated by their physiological properties and is independent of their immunological specificity (Economou et al, 1996). NK cell populations may have high affinity for tumor vessel regions, but may be limited to a single passage through the tumor vasculature due to entrapment in other organs. In contrast, T lymphocytes may have lower adhesion efficiency to tumor vessels, but are not limited to single-pass delivery (Jain, 2001). Due to these features, several groups are studying improvement of T cell tumor homing. The approach consists mainly of construction of chimeric receptors encoding a peptide or protein able to recognize tumor antigens. The extracellular moiety of this artificial receptor is generally a single-chain antibody or ligand of endothelial and/or tumor cell

receptors. Alternatively, the TCR can be modified, although this requires functional assembly with the endogenous signaling machinery (Goverman et al, 1990; Brockner et al, 1996). Other groups have incorporated an intracellular domain that allows certain activation signals to promote rolling or immune functions (Hombach et al, 2002). Engineering T cells is a promising strategy, but efficacy in clinical trials remains to be demonstrated.

2. Macrophages

Macrophages are phagocytic cells distributed throughout the body. Under normal conditions, they circulate without tissue retention, but under pathological circumstances they are mobilized and concentrated in damaged areas. Low oxygen tension (hypoxia) is another signal for macrophage recruitment, for example to areas of tumor necrosis (Goerdts et al, 1999).

A significant proportion of cells in tumors are macrophages, apparently rendering them good candidates for cell-based therapies (Ohno et al, 2002). The ability of macrophages to kill tumor cells is controversial. Some groups have reported that activated macrophages kill tumor cells by direct cytotoxicity and by antibody-dependent cytotoxicity, although others question the reality of these activities. Activated macrophages could kill tumor cells by secreting superoxide anions, hydrogen peroxide, nitric oxide or proteolytic enzymes, although tumor cell destruction by macrophages requires cell-to-cell contact and is dependent on contact duration, target cell type, and other poorly understood mechanisms (Obenig, 1997).

Previous studies showed the safety of protocols based on the infusion of large numbers of macrophages into patients. Activated macrophages were effective in treatment of metastases, around which they accumulated; in contrast, primary tumors did not regress (Fidler et al, 1985). Once their tumor homing capacity has been demonstrated, macrophage efficacy could be improved if they carried therapeutic genes to be expressed near or within a tumor. Engineered receptors and hypoxia-regulated promoters have been used to obtain higher specific targeting in some vectors in preclinical models (Griffiths et al, 2000).

3. Dendritic cells

Dendritic cells are bone marrow-derived antigen-presenting cells able to migrate to local lymph nodes, where they activate T cells. In addition, the presence of tumor-infiltrating dendritic cells (TIDC) in a tumor has been correlated as a good prognostic factor for several tumor types (Bell et al, 1999). TIDC capture and process tumor cell-derived antigens, then migrate to lymph nodes to activate anti-tumor immune responses (Randolph et al, 1999).

Early clinical trials involving immunization of patients with dendritic cells are now in progress. These trials show considerable variation as to the source of these cells and their route of administration, although all are based on an immunization strategy (Mulé, 2000). Little

information is available about TIDC use in cell-based local therapies.

An indirect approach is based on injection into tumors of gene-modified TIDC expressing IFN- γ . This chemoattractant promotes a sustained T cell influx into the tumor mass, potentially improving therapeutic efficacy (Kirk et al, 2001). Zou et al, (2001) defined a dendritic cell subpopulation specifically recruited to the tumor microenvironment, and reported that local regeneration or proliferation were not important mechanisms for accumulation. They also suggested a role for VLA-5 in migration to tumor tissue, and speculated that SDF-1 may be the major tumor-related chemoattractant for dendritic cells. This dendritic cell subpopulation may thus be a good candidate for loading with therapeutic agents to target the tumor microenvironment.

B. Tumor cells

The use of tumor cells as anti-tumor agents is based on several observations suggesting their potential utility. In their respective models, Coukos et al, (1999) and Namba et al, (1998) reported that infused tumor cells bind preferentially to tumor masses of the same histological type. When they loaded a tumor cell carrier line with a therapeutic vector, significant reduction of pre-existing tumor size was observed, although their models were based on tumors implanted in localized areas such as brain and peritoneal cavity.

Some researchers have developed a model of spontaneous metastasis, with which they hypothesized that intravenously (IV) injected tumor cells would home to the organs with metastases (Garc_a-Castro et al, unpublished data). Metastases arise from local growth of malignant cells that have separated from the primary tumor, reached the blood and/or the lymphatic circulation and localized in distant organs. In this process, tumor cells traveling in the bloodstream respond to factors produced by or present in the different organs, an interaction that results in a specific metastatic pattern for each tumor. Genetically transduced tumor cells injected IV localize in pre-existing metastatic lesions, and demonstrated that cells carrying an antitumor agent such as a suicide gene or an oncolytic virus could deliver a localized therapeutic effect with negligible systemic toxicity.

In vitro experiments using three-dimensional matrices suggested that invading tumor cells leave signals in their wake that drive migration of other cancer cells into the matrix (Horino et al, 2001). It is interesting that the cells following the invasive front in the matrices had a non-invasive phenotype. Tumor cells injected IV would thus participate in this interaction with the microenvironment, and target invading metastases independently of their invasive capacity. Although our experiments were performed with autologous tumor cells, it is possible that the cell carrier could be non-autologous to the patient, and a standard tumor cell line could be even developed with the aim of simplifying use in clinical trials.

C. Stem cells

Pluripotent stem cells, derived from human fetal tissues, have the ability to differentiate into almost any cell type found in embryonic germ layers. It was believed until recently that adult organ-specific stem cells were lineage-restricted, but recent studies have questioned this idea, and multipotent stem cells have been found in many adult tissues (Preston et al, 2003).

Several groups have reported that some stem cells can target tumors and differentiate into diverse cell types, particularly into blood vessel endothelial cells (Carmeliet, 2003). Tumor growth requires neovascularization, and tumors thus promote remodeling of pre-existing capillaries and mobilization of bone marrow-derived cells through secretion of VEGF and other angiogenic factors (Bergens and Benjamin, 2003). As there are few active angiogenesis sites in a healthy adult, this capacity to home to the tumor site could be used to deliver therapeutic agents following *ex vivo* manipulation of adult stem cells.

Endothelial progenitor cells (EPC) are highly proliferative cells derived from bone marrow; at difference from mature, differentiated circulating endothelial cells, they are incorporated into new tumor vessels (Asahara et al, 1999). Certain hematopoietic cell subsets also contribute to angiogenesis. Human bone marrow-derived EPC, inoculated into tumor-bearing immunodeficient mice, were detected in newly-formed tumor vessels, although with considerable variation among animals. This finding provides additional justification for the use of EPC and/or bone marrow stem cells as carriers in cell-based anti-tumor strategies. This approach has been used by several groups to express angiogenesis inhibitors or suicide genes in mouse models; they inhibited tumor growth and prolonged animal survival (Rafii et al, 2002; Ferrari et al, 2003). A conditioning regime (such as bone marrow transplantation), nonetheless, seems to be necessary for the success of the experiments (Lyden et al, 2001), probably because irradiation suppresses endogenous EPC and/or enhances EPC uptake into tumor vasculature.

De Palma et al, (2003) recently reported a bone marrow-derived population that homes to tumors and interacts closely with vascular endothelial cells. This population was termed Tie-2-expressing mononuclear (TEM) cells, as their description is based on expression of a marker gene directed by a Tie-2 promoter. After transplant of hematopoietic cells transduced with Tie-2 promoter-lentiviral vectors, several tumors were injected and their angiogenesis analyzed. TEM expressed CD45 and CD11b, and were associated with small blood vessels assembled into a stromal framework in close association with endothelial cells. Finally, TEM were transduced with a suicide gene; tumor-bearing animals treated with the pro-drug showed delayed tumor appearance and slower tumor growth (De Palma et al, 2003). TEM cells thus appear to be a suitable vehicle for tumor-directed cell therapy.

Mesenchymal stem cells (MSC) are the progenitors of several mesenchymal lineages, are present in various tissues and can be expanded in culture without losing their

phenotype or multilineage potential (Minguell et al, 2001) (**Figure 3**). Although MSC are currently recovered from bone marrow, they are distinct from hematopoietic stem cells and have a different expression marker profile. Moreover, MSC can be isolated from muscle, cord blood, cartilage or adipose tissue (Young et al, 2001; Zuk et al, 2001). MSC can be used in therapeutic strategies, as they can target organs (especially when implicated in pathological cases) and are easily transduced with viral vectors. *In vivo* transgene expression was reported following transplant of retrovirally transduced MSC (Deans and Moseley, 2000). Studeny et al, (2002) reported that when MSC were administered IV into mice with melanoma, these cells were detected inside tumors as stromal fibroblasts. In addition, survival was prolonged when engineered MSC were induced to secrete IFN . There is a pluripotent cell population that co-purifies with MSC, termed multipotent adult progenitor cells (MAPC). A single MAPC injected into an early blastocyst contributes to mesodermic, neuroectodermic and endodermic tissues (Jiang et al, 2002). MAPC are able to differentiate into endothelial cells *in vitro* and *in vivo*. *In vitro*-generated MAPC-derived endothelial cells respond

to angiogenic stimuli by migrating to tumor sites and contributing to tumor vascularization (Reyes et al, 2002). These characteristics indicate that both MSC and MAPC could thus be used as therapeutic carriers to express anti-tumor factors at the site of neoplasms.

Pharmacological agents for tumors derived from cells of the central nervous system must cross the blood-brain barrier; due to the immune-privileged nature of this tissue, the use of cellular vehicles might overcome this problem. Previous studies using fibroblasts, myoblasts, macrophages and endothelial cells (Schinstine et al, 1991; Jiao et al, 1992; Messina et al, 1992, Lal et al, 1994) encountered specific problems, as they were limited to the injection site due to the lack of motility or potential physiology problems because they are not normal brain components. Nonetheless, a new strategy using neural stem cells (NSC) has recently been reported. NSC have exceptional migratory ability; Aboody et al, (2000) showed that NSC can target invasive primary brain tumors, a behavior not displayed by cells of non-neural origin.

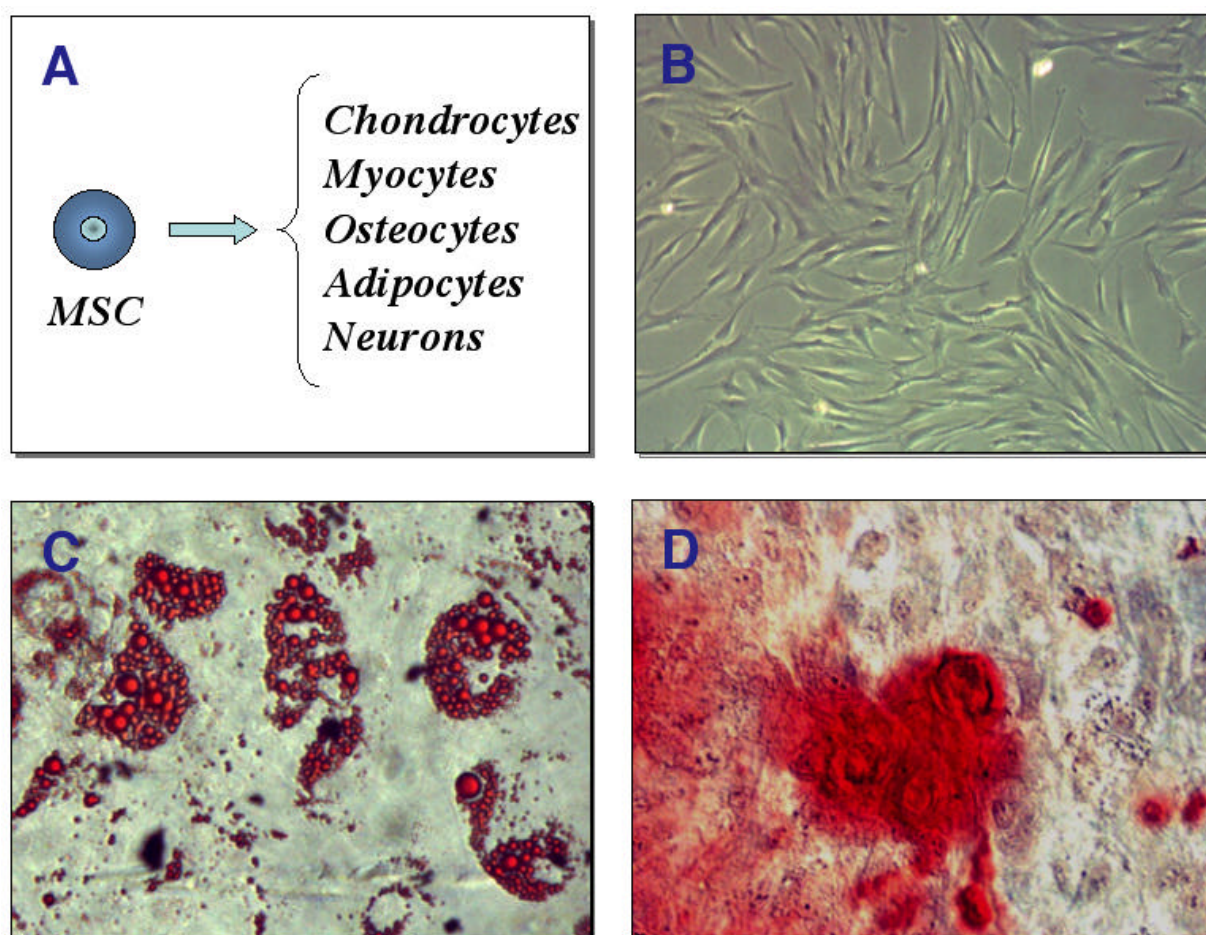


Figure 3. Differentiation potential of mesenchymal stem cells. (A) Mesenchymal stem cells (MSC) have the capacity to differentiate, at least, into osteoblasts, chondroblast, myoblast, adipocytes and neurons when they are cultured in the induction medium and certain substances are added. (B) Appearance of human bone marrow-derived MSC after several days of culture. (C) Oil Red staining of human MSC after two weeks of culture in adipogenic differentiation medium. Lipid droplets are staining in red. (D) Alkaline phosphatase detection (in red), indicating an osteogenic differentiation of human MSC.

These authors also showed reduction of established tumors when suicide gene-transduced NSC were used as a therapeutic approach. NSC also appear to follow infiltrating tumor cells that escape from normal tissue, although no reports have yet described the possibility that NSC injected into systemic circulation target intracerebral tumors. Herrlinger et. al, (2000) used replication-conditional vectors to transduce NSC and observed distribution throughout a glioma tumor. These studies thus indicated that NSC may provide a powerful cell-based antitumor therapy.

III. Conclusions

The use of cells as carriers for antitumor agents is a very attractive therapeutic strategy, although many aspects of this approach remain to be elucidated. Further knowledge is, nonetheless, needed of specific tumor-targeting mechanisms and the *in vivo* physiological behavior of in each carrier cell type. Preclinical researchers must define the properties of these cells and find ways of manipulating them to produce a clinically appropriate outcome. Quality control is necessary in the manufacturing process as well as of the final product. Clinicians will need to adapt therapeutic regimens in accordance with the biology of these cells, their route of administration and the dose to be employed. In conclusion, within a few years these "Trojan horse" cells may offer an alternative therapeutic strategy for certain tumor types.

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