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Genetically modified mesenchymal stromal cells in cancer therapy

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Abstract

The cell therapy industry has grown rapidly over the past 3 decades, and multiple clinical trials have been performed to date covering a wide range of diseases. The most frequently used cell is mesenchymal stromal cells (MSCs), which have been used largely for their anti-inflammatory actions and in situations of tissue repair and although they have demonstrated a good safety profile, their therapeutic efficacy has been limited. In addition to these characteristics MSCs are being used for their homing and engraftment properties and have been genetically modified to enable targeted delivery of a variety of therapeutic agents in both malignant and nonmalignant conditions. This review discusses the science and technology behind genetically modified MSC therapy in malignant disease and how potential problems have been overcome to enable their use in two novel clinical trials in metastatic gastrointestinal and lung cancer.

Introduction

The landscape of cellular therapies has changed dramatically over the past 20 years and is likely to continue to do so over the next decade. There is an increasing drive to overcome existing roadblocks to large-scale use to provide a more streamlined route to market. The value of the cell therapy industry is projected to reach £20 billion by 2022, and the array of cell therapies being investigated is rapidly expanding [1]. There are currently more than 500 clinical trials using mesenchymal stromal cells (MSCs) registered on the National Institutes of Health clinical trials database and an increasing proportion of these are using genetically modified MSCs (http://www.clinicaltrials.gov; accessed August 2016). In the United Kingdom alone 37% of trials use genetically modified cells, the majority of which use viral vectors for gene delivery [2].

The term "cell therapy" covers a wide array of products, and they are most commonly classified according to cell type (e.g., hematopoietic stem cells, MSCs, embryonic stem cells, modified T cells). Within these cell types, the range of diseases being treated are vast, ranging from immunomodulation to target inflammatory diseases such as inflammatory bowel disease [3], chronic obstructive pulmonary disease [4] and acute lung injury [5,6] to acute stroke [7,8], acute myocardial infarction [9,10], and graft-versus-host disease [11]. Stem cells are also being used for tissue repair and regeneration with differentiation being directed to the target organs such as bone and cartilage [12]. There is also increasing interest in the use of genetically modified cell therapies including chimeric antigen receptor (CAR) T cells and cells genetically modified to express therapeutic proteins targeted to a specific disease.

Within this review we discuss the use of genetically modified MSCs as a therapy for cancer and in particular discuss our own experience of developing and cell and gene therapy product for the treatment of metastatic lung cancer to be delivered in a phase I/IIa clinical trial.

MSCs

MSCs were first described in the 1970s by Friedenstein *et al.* [13] and are now one of the most widely characterised adult stem cells. As determined by the International Society for Cellular Therapy (ISCT), they must meet the minimum criteria of being adherent to tissue culture plastic under standard culture conditions, express the cell surface markers CD105, CD73 and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. In addition, they must be capable of differentiating into adipocytes, osteoblasts and chondroblasts under the correct experimental conditions [14]. MSCs are a heterogeneous population of cells, and their characteristics are affected by passage, cell density and culture conditions [15]. They are

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readily available from multiple sources, including bone marrow [16], adipose tissue [17] and umbilical cord [18], among others, and although cells from all sources will meet the minimum criteria for MSC definition, there are subtle differences in their behavior that further complicates our understanding of this cell type. These differences may be therapeutically beneficial in some cases in terms of either their secretory profile or growth characteristics, but as yet there are no data that directly compare the core characteristics of the different sources of MSCs, and the ideal cell source is likely to be dependent on the indication for its use.

MSCs can be easily extracted from adults and expanded *in vitro* and, once isolated, have a number of characteristics that make them appealing vectors for delivery of therapeutic agents. One of the key properties of MSCs is their tumor tropism, that is, their propensity to move toward sites of tumor [19,20]. The precise mechanism through which this process occurs is unknown, but it has been demonstrated in multiple cancer models including glioma [21,22], breast carcinoma [23], lung cancer [24,25], malignant mesothelioma [26], hepatocellular carcinoma [27,28], colon cancer [29], pancreatic cancer [30,31], ovarian cancer [32], melanoma [33] and Kaposi sarcoma [34]. The tropism is thought to be mediated through paracrine signaling between the tumor microenvironment and corresponding receptor expression in MSCs. Although tumor tropism has been consistently demonstrated, the precise mechanisms responsible remain poorly understood. Many factors have been assessed with regards to this property including multiple receptors, extracellular matrix proteins, tumor necrosis factor α (TNFα), interleukins (ILs), macrophage migration inhibitory factor (MIF) and, most frequently, the soluble tumor–derived factor stromal-derived factor (SDF)-1 [35–37]. The most widely studied interaction has been that between SDF-1 and CXCR4, but the involvement of this axis remains controversial [38].

Another characteristic of MSCs that make them attractive for therapeutic use is their low immunogenic state in that they elicit a weak allogeneic immune response when delivered to a non-identical, non-matched recipient [39,40]. These unique properties are attributed to the low levels of expression of major histocompatibility complex (MHC) class I and the costimulatory molecules CD80 and CD86 and the lack of MHC class II proteins [41–44]. Because of these properties, there is the potential for using allogeneic MSCs as an "off-the-shelf" product. To use cells from healthy, young donors that are likely to have greater regenerative capacities and higher proliferative rates would be an attractive option to help control the costs and complexity of the manufacturing process, which would be a significant factor in the long-term likelihood of making cell therapies commercially viable. Although there is evidence that the source of MSCs and their culture conditions can alter their immunomodulatory properties, there is no direct comparison of the immune profile of cells from different sources or after culture in different conditions [45,46].

Genetic modification

Going hand in hand with their tumor tropism is the ability of MSCs to be modified to allow sustained delivery of specific anti-cancer agents. Because the cells are attracted to tumor stroma, targeted therapeutic delivery can be achieved at multiple tumor sites. There are many methods to genetically modify MSCs, but they can be broadly classified into viral and non-viral methods. A detailed discussion of methods of modification and MSC engineering is outside the scope of this review; however, excellent overviews of this are provided by Park *et al.* [47] and others [48,49].

Non-viral vectors

Non-viral methods of gene transfer encompass all physical and chemical methods of gene delivery. These methods are appealing because they are able to deliver larger transgenes than viral methods, are more cost-effective and are amenable to scale-up manufacturing and induce less of an immune response. Despite these benefits, there are a number of limitations, the main one being their low transfection efficiencies and transient gene expression [47]. Physical methods of gene delivery are based on temporarily penetrating the cell membrane using techniques such as electroporation [50–53], ultrasound [54,55], magentofection [56] and DNA particle bombardment by gene gun [57,58]. Chemical methods tend to use cationic lipids or polymers, which form negatively charged particles that are taken up into the cell by endocytosis, but these methods are largely limited to *in vitro* use [59,60]. Cell surface receptors have been explored, and other non-viral methods of modification being investigated are via liposomes [61] or nanoparticles [62].

Viral vectors

Viral transduction of MSCs is commonly achieved using lenti-, retro-, adeno- or adeno-associated virus without affecting their stem cell properties [63,64]. Viral vectors use the innate ability of the virus to gain entry into and survive within the host cell nucleus to ensure continued expression of the viral genome. To make them useful as delivery vectors, they have undergone significant modification to produce replication incompetent viruses with attenuated cytopathic effects and immunogenicity. One of the enduring concerns regarding the use of viral vectors is their safety, but